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This book composes the abstracts of the presentations for the platform and poster sessions of the 25th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at the International Congress Centre Barcelona (CCIB), in Barcelona, Spain from 3-7 May 2015. The abstracts are reproduced as accepted by the scientific committee of the meeting and appear in order of abstract code, in alphabetical order per presentation type. The poster spotlight abstracts are included in the list of poster abstracts. The presenting author of each abstract is underlined.
In the 1970s, no forum existed for interdisciplinary communication among environmental scientists—biologists, chemists, toxicologists—as well as managers and engineers others interested in environmental issues. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void. Based on the dynamic growth in the Society’s membership, meeting attendance and publications, the forum was clearly needed. SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1990, and in Brussels, Belgium, established in 2003.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers, World Council, Geographic Unit Boards of Directors and Councils, and Committee members and governance of activities. The proportion of members from each of the three sectors has remained nearly equal over the years.

SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.

The Society is concerned about global environmental issues. Its members are committed to Environmental Quality through Science®, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. SETAC’s growth has been marked the establishment of geographic units around the world: SETAC Europe in 1989, SETAC Asia/Pacific in 1997, SETAC Latin America in 1999 and SETAC Africa in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters of the geographic units are being considered for a number of countries.

Publications
Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1980 to a monthly publication of nearly 3,000 pages annually.

Integrated Environmental Assessment and Management, launched in 2005 to bridge the gap between scientific research and it application in environmental decision-making, regulation and management, has become a well-respected quarterly publication of 700 pages annually.

SETAC Books total more than 100, encompassing workshop results and other scientific studies.
Keynote Abstracts

K1 Then, now……and beyond; some personal reflections on 25 years of the Society and its science.
Peter Calow, Office of Research, University of Nebraska-Lincoln, USA.
Having been involved in the foundation of SETAC in Europe, Peter Calow will offer a personal take on how the science has developed over the past 25 years together with some stories about his interactions with the Society and its members. One aspect of the development of the science has been a shift from concerns about reliability and repeatability to an increasing preoccupation with the somewhat elusive concept of relevance. From Sheffield to Barcelona annual meetings have enhanced these issues and ensured that they have been given due attention. As we go forward and grapple with making even more of a difference for public environmental policy, the future may well invite a broader scope for our collaborations including, for example, the social sciences and economics.

K2 Towards a big data-driven solution for cooperative and effective management of chemical risks
John K. Colbourne, School of Biosciences, University of Birmingham, UK
EU lawmakers have taken the bold step of legislating the need to assess the toxicity of all chemicals sold in Europe (REACH). This has spurred science to provide a robust and cost-effective solution. However, there are huge scientific constraints in determining the environment and human health risks for an ever-increasing number and diversity of consumer products, while toxicity testing fails to keep pace with modern biology. This has created an enormous backlog of chemicals that have yet to be assessed for potential health hazards.
Considering the scale of the current problem, and realistic future projections, a shift to next-generation assays is both timely and necessary. The proposed solution is Phylogenetic Toxicology, which applies high-throughput toxicity testing with data-rich genomics assays applied to 3R compliant model species representing animal biology. These include biomedical and ecologically relevant organisms that altogether can deliver experimentally derived predictions of a chemical’s modes of actions and key events in the etiology of illness or injury. This solution to chemical risk assessment incorporates an important discovery made within the past 10 years in studies of the functional elements in animal genomes; a significant suite of elements and their functional associations for growth, maintenance and reproduction is shared among animals (including humans) representing over 60% of transcribed and epigenetically modified genomes. This crucial finding is reinvigorating the use of experimental, scientifically and legally accepted biomedical alternative model species for understanding the human condition. This finding also provides a proven platform for the necessarily big and transformative set of experiments that combine genomics, metabolomics, evolutionary theory, bioinformatics and toxicology to meet the regulatory challenges of today and tomorrow.
Ultimately, the chemical safety reporting practice of tomorrow should be based on key events described within adverse outcome pathways (AOPs) and predicted to be shared with humans and other untested species using statistical and evolutionary principles. Future safety testing of chemicals will therefore be built upon obtaining prescribed types of useful information, instead of following prescribed methods of satisfying regulatory requirements, which is made inexpensive by enabling technologies.
This talk is delivered on behalf of the Consortium for Environmental Omics and Toxicology.

K3 Wastewater derived contaminants of emerging concern. Current and future challenges
Mira Petrovic , a, b
a Catalan Institute for Water Research (ICRA), Girona, Spain
b Catalan Institution for Research and Advanced Studies (ICREA) , Barcelona, Spain
Treatment of wastewaters from domestic and industrial practices is of crucial importance in modern society. The increase in urban population, changing lifestyles and industrialization results in increase quantity of wastewater that requires treatment before it can be discharged into the aquatic environment or reused for any purpose. In parallel, the use of chemicals by our technological society is continuously growing and currently can be estimated in some hundreds of thousands of compounds (most of them organics) in daily use. Since the early 1970s municipal wastewater treatment plants (WWTP) were basically designed to remove pathogens, nutrients and organic and inorganic suspended and flocculated matter, but not microcontaminants. Since 1980, the rise of health concerns related to microcontaminants has driven the development of new treatment technology (biotic and abiotic membrane treatments, advance oxidation and reduction processes, electrochemical treatments, combined processes etc). However, in spite of a range of advanced treatment options available, at urban WWTP typically using secondary biological treatment (CAS – conventional activated sludge) not all organic contaminants are removed and discharge of treated effluents result in degraded receiving water quality. Therefore, WWTP effluents are considered the principle route of entry of many microcontaminants into the environment. Most concerning are polar compounds that are, due to their physico-chemical properties (high water solubility and often poor degradability), able to penetrate through all natural filtration steps and man-made treatments, thus presenting a potential risk in drinking water supply. Many of these compounds originate from consumer products used in households or from industry and are generally not regulated (i.e. emerging contaminants).
This work will overview current and future challenges related with the presence of microcontaminants of emerging concern in wastewaters, such as the need to increase our knowledge about the fate of emerging microcontaminants during sewage treatment; cumbersome task of the identification of degradation products and understanding their contribution to the risk posed by emerging contaminants; risk of understudied and/or never targeted compounds; potentials and feasibility of advanced treatments; and the occurrence of emerging contaminants in environmental waters in relationship to their removal in WWTP.
Platform Abstracts

Ecological Consequences of Exposure to Pharmaceuticals (I)

1 Ecopharmacovigilance of human medicinal products: How it works in practice
J.R. Snape, AstraZeneca UK Ltd.; AstraZeneca Global Environment; R. Murray-Smith, AstraZeneca; C. Coleman, AstraZeneca / Brixham Environmental Laboratory; N. Budgen, AstraZeneca / Essential Safety Health and Environment; P. Sorne, AstraZeneca / AstraZeneca Patient Safety

Ecopharmacovigilance (EPV) is a developing science concerned with the detection, evaluation, understanding and prevention of adverse effects of active pharmaceutical ingredients (APIs) in the environment. In Europe and North America, a new regulatory submission or a life extension is normally accompanied by an Environmental Risk Assessment (ERA), but there is no regulatory requirement to monitor the environmental risks of medicines post-launch. Nevertheless, researchers globally continue to report instances of pharmaceutical residues in the environment, and publish new ecotoxicological studies on effects of pharmaceuticals on wildlife. To understand the significance of these emerging data and ensure that any potential risks are identified and managed appropriately, we have developed a process for EPV. This ensures that we can continue to understand the environmental risks associated with our products, and to manage them appropriately throughout the life of each product. This presentation will describe our EPV processes, describe how it works in practice and demonstrate some of the significant findings we have made to date.

2 Exploiting monitoring data in the spatial modellling of exposure of ecosystems and consumers to pharmaceuticals in the environment
A. Boxall, University of York / Environment Department; R. Williams, Centre for Ecology and Hydrology; V. Keller, Centre for Ecology & Hydrology; S. Monteiro, APC Ltd.; R. Fussell, Food and Environment Research Agency; J. Straub, F.Hoffmann-La Roche Ltd / Roche Group Safety Health Environmental Protection

In order to establish the impact of residues active pharmaceutical ingredients (APIs) in the environment on ecosystem and human health, good information on the level of exposure in surface waters and drinking waters is needed. Exposure concentrations are typically estimated using information on the use of an API as well as removal rates in the patient, the wastewater system and in surface waters. These input data are often highly variable and difficult to obtain, so model estimates often do not agree with measurements made in the field. In this study we used an inverse modelling approach to estimate overall removal rates of pharmaceuticals at the catchment scale using a hydrological model as well as prescription and monitoring data and available monitoring data. Use of the approach, alongside estimates of predicted no-effect concentrations for the 12 study compounds, to assess risk of the APIs across the UK landscape indicated that, for most of the compounds, risks to aquatic life were low. However, ibuprofen was predicted to pose an unacceptable risk in 50% of the river reaches studied. For diclofenac, predicted exposure concentrations were also compared to the Environmental Quality Standard previously proposed by the European Commission and 4.5% of river reaches were predicted to exceed this concentration. Risks to human health were also predicted to be very low. While the current study focused on pharmaceuticals, the approach could also be valuable in assessing the risks of other ‘down the drain’ chemicals and could help inform our understanding of the important dissipation processes for pharmaceuticals in the pathway from the patient to ecological receptors.

3 Diclofenac: 40 years story of health benefits and environmental concerns. A case study on regulatory inconsistencies
V. Acuña, RiE; A. Ginebreda, CSIC - Spanish National Research Council / Department of Environmental Sciences; M. Petrovic, ICRA; J.R. Mor, Catalan Institute for Water Research ICRA; s. sabater, Universitat de Girona; J.P. Stumpfer, Brunel University / Institute for the Environment; D. Barcelo, IQIAB-CSIC / Institute for Environmental Assessment and Water Research

Our developed technical society has been capable to design, and produce a great variety of molecular entities for improve human wellbeing. However, even the most useful and beneficial molecules might exhibit a “dark side” when placed out of its original “focus”. The policies necessary to modulate such delicate balance between these opposite aspects are provided by the diverse existing regulations, but there is an increasing public perception that regulations follow a ‘reactive’ rather than ‘proactive’ behavior. The aforesaid situation can be well illustrated with the example of Diclofenac. To illustrate the two sides of this particular molecule (effects on human wellbeing and on the environment), we reviewed the scientific literature to identify all scientific publications dealing with Diclofenac since its launch in 1975, and found that most of these publications focus on human health (81.9 %). Diclofenac has been widely used for human health purposes since the 1970s. National annual consumptions of Diclofenac corresponding to the period 2010-2013 range from 2 to 60 Tons, and the global annual consumption was of 43 55 Tons. Regarding occurrence ecotoxicological, Diclofenac has been reported to occur in many freshwater ecosystems around the World, and median concentrations of the world nations averaged 58 ± 162 ng L^{-1}. The comparison of global maps of consumption and occurrence reveals that data on Diclofenac occurrence in freshwaters is poor or absent in large parts of the globe where there is consumption and most presumably occurrence in freshwaters. Molecules reaching the environment are developed under the application of strict sectoral legislations (medicines, plant protection products, industrial etc.). These pieces of legislation nowadays usually take into consideration severe environmental and human health requirements as well. Contrastingly, environmental legislation is horizontal rather than sector related, and what is more relevant, it typically works “ex post”. Furthermore, the time lag between the appearance of scientific evidences on harmful effects on the environment and the regulation of molecules with even small effects on human wellbeing is, in our opinion excessive if compared to the evolution of scientific knowledge, and somehow resembles the period of time elapsed from the publication of the Silent Spring, and the banning DDT worldwide.

4 Top-down and bottom-up effects in a diclofenac contaminated model ecosystem
S. JOACHIM, INERIS / CIVS; R. Beaudouin, INERIS / Models for Ecotoxicology and Toxicology METO; E. Vulliet, CNRS; A. Bado-Nilles, UMR SEBIO; G. Daniele, ISA / Equipe Traces UMR; L. Chabot, INERIS / EXES; P. Baudoin, INERIS / ECR 55; O. Paluel, INERIS / Université de Savoie; C. Jenkins, AstraZeneca / AstraZeneca Patient Safety

Ecopharmacovigilance (EPV) is a developing science concerned with the detection, evaluation, understanding and prevention of adverse effects of active pharmaceutical ingredients (APIs) in the environment. In Europe and North America, a new regulatory submission or a life extension is normally accompanied by an Environmental Risk Assessment (ERA), but there is no regulatory requirement to monitor the environmental risks of medicines post-launch. Nevertheless, researchers globally continue to report instances of pharmaceutical residues in the environment, and publish new ecotoxicological studies on effects of pharmaceuticals on wildlife. To understand the significance of these emerging data and ensure that any potential risks are identified and managed appropriately, we have developed a process for EPV. This ensures that we can continue to understand the environmental risks associated with our products, and to manage them appropriately throughout the life of each product. This presentation will describe our EPV processes, describe how it works in practice and demonstrate some of the significant findings we have made to date.

5 Reproductive and endocrine effects of mixtures of steroid pharmaceuticals with diverse mechanisms of action in a fish reproduction assay
T.J. Thunnin, Brunel University / Institute for the Environment; T.J. Runnalls, Brunel University / IFE; G.C. Parnell, London Metropolitan University / Institute for the Environment; S. Kaguthas, Brunel University / Institute for the Environment; A. Kortenkamp, Brunel University; J.P. Stumpfer, Brunel University / Institute for the Environment

The widespread environmental occurrence of human pharmaceuticals in effluents, surface waters, seawaters and groundwater has increased concern for potential endocrine disrupting effects and potential endocrine disruptor activity of pharmaceuticals. These pieces of legislation are biologically active and might be persistent, toxic effects on organisms are suspected at low concentrations and have been demonstrated mainly for steroid hormones. Diclofenac is a non-steroidal anti-inflammatory drug, which is found at concentrations ranging between 10-100 µg/L in European freshwaters ecosystems. As chronic ecotoxicity data as well as information on the current distribution patterns in environmental compartments are scarce, an integrated hazard assessment strategy was required to allow the development of low tier test models (e.g. ecotoxicity test, biomarkers studies) and high tier studies (loxic mesocosms). The results of the loxic mesocosm experiment are described in this communication. The study was carried out, in twelve 20 meter long channels, under continuous, environmentally realistic concentrations of diclofenac (0.1, 1 and 10 µg L^{-1} in triplicates). The mesocosms were set up with artificial sediments, macrophytes, periphyton, benthic and pelagic invertebrates, decomposers and one fish species (Gasterosteus aculeatus). After three months of ecosystem stabilization, treatment lasted 6 months. Periphyton biomass, macrophyte biovolume, zooplankton and invertebrate abundance and diversity and fish individual physiological responses along with population dynamics were the measured biological endpoints. Diclofenac concentration in water was monitored each month by chemical analysis. In order to account for the observed effects, degradation products and major metabolites were also measured in the sediment, watercress and fish. Effects on water quality parameters, macrophytes and fish were observed at the highest tested concentration. Top-down and bottom-up effects seem to drive and structure the responses of the other populations and communities. A conceptual model presenting the overall response is proposed.

5 Reproductive and endocrine effects of mixtures of steroid pharmaceuticals with diverse mechanisms of action in a fish reproduction assay
T.J. Thunnin, Brunel University / Institute for the Environment; T.J. Runnalls, Brunel University / IFE; G.C. Parnell, London Metropolitan University / Institute for the Environment; S. Kaguthas, Brunel University / Institute for the Environment; A. Kortenkamp, Brunel University; J.P. Stumpfer, Brunel University / Institute for the Environment

The widespread environmental occurrence of human pharmaceuticals in effluents, surface waters, seawaters and groundwater has increased concern for potential endocrine disrupting effects and potential endocrine disruptor activity of pharmaceuticals. These pieces of legislation are biologically active and might be persistent, toxic effects on organisms are suspected at low concentrations (ng/l range), such as impairment of reproduction. Key questions for chemical risk assessment are whether steroid pharmaceuticals can produce adverse effects on organisms when present in the environment in combination and at low concentrations, whether these can be classified as additive, and under what circumstances can they be predicted. A fish reproduction assay was used to determine reproductive and endocrine effects of five steroid pharmaceuticals. A
series of in vivo experiments were carried out for single steroid compounds using a well-established 21 day pair-breeding assay. Adult fathead minnows (Pimephales promelas) were exposed to steroidal pharmaceuticals from four different drug groups; estrogens, progestins, androgens and glucocorticoids. Each compound tested was found to decrease egg production in a concentration-dependent manner. A mixture study was subsequently designed based on mathematical modelling of single compound data. Using regression modelling and the mixture prediction models Concept for Chemical Action (CA) and Independent Action (IA), a multicompound mixture experiment was designed based on a fixed equipotent mixture ratio of the five compounds. Three mixture concentrations were tested in the reproduction assay. Effects of the mixture on egg production showed a clear concentration response mixture relationship. At the lowest mixture concentration, where individual chemicals were present each at concentrations that were estimated to produce only minor effects well below the statistical detection limit of the study design, a clear combination effect was observed (ca. 40% egg reduction). The work presented here suggests that pharmaceuticals present in ‘real world’ mixtures have the potential to act additively on certain organisational levels at low concentrations, and that combination effects should be accounted for in risk assessments.

6 Developmental exposure to progestins cause male bias and precocious puberty in zebrafish (Danio rerio)

J. Svensson, Uppsala University / Department of Environmental Toxicology; A. Mustafa, Uppsala University / Department of Comparative Physiology; I. Brandt, Uppsala University / Department of Environmental Toxicology; M. Schmitz, Uppsala University / Department of Comparative Physiology; B. Brunström, Uppsala University / Department of Environmental Toxicology Progestins are environmental contaminants which impair reproduction in fish at low concentrations. The mechanisms behind this effect have not yet been elucidated, but are likely multiple since different progestins activate not only progesterone receptors (PRs) but other steroid receptors as well. We previously showed that progestins at relevant concentrations of emerging contaminants in adult fish and zebrafish model species in environmental testing are pending, and that combination effects should be accounted for in risk assessments.

7 Fish model species in environmental toxicology (I)

7 Use of “Omics” approach to assess adverse impact of environmentally relevant concentrations of emerging contaminants in adult fish and zebrafish embryos

H.R. Habibi, University of Calgary / Department of Biological Sciences; A. Zare, Department of Biological Sciences; J. Jordan, University of Calgary / Biological Sciences; C. Kinch, University of Calgary / Department of Biological Sciences; D.M. Kurrasch, University of Calgary / Department of Medical Genetics; A. Weljie, University of Pennsylvania / Department of Pharmacology Contaminants of emerging concern (CECs) are known to pose health risk to animals and humans. However, insufficient information is available about the mechanisms by which these compounds cause adverse physiological and pathological effects. We have previously observed significant increases in the male-to-female ratio in wild populations of longnose dace caught down stream of municipalities along the Oldman River, Canada. We performed controlled laboratory experiments in which adult and embryonic fish were exposed to low, environmentally-relevant concentrations of a selected number of chemicals detected in the river system, individually and as mixtures. Physiological, morphometric, transcriptomics and metabolomics approaches were used to investigate the mechanisms by which CECs disrupt reproduction and development in fish. Metabolomics studies demonstrate significant dysregulation of amino acid, lipid, energy, carbohydrate, nucleotide and cofactor/vitamin metabolism. Exposure of fish to mixture of chemicals resulted in relatively unique pattern of metabolism distinct from individual contaminants in gonad, liver and brain. In separate experiments, exposure of fathead minnow to environmental contaminant mixtures resulted in significant changes in transcriptome profiles, using well-characterized microarrays. We identified candidate low-dose biomarkers of chemical exposure in fish, and in combination with metabolomics data, the results provide critical information on cellular response following exposure to CECs. In zebrafish, exposure of developing embryos to contaminants resulted in precocious neurogenesis via an androgen receptor and Aromatase-B mediated mechanism, linking neurodevelopmental effects with sex as a consequence of changes in timing of neuronal birth. The results of our studies can be used to assess risk through mechanism-based understanding of cell and tissue response. The overall results support the hypothesis that different chemicals present in the Oldman River disrupt fish health by altering gene expression profile and metabolism of the liver, ovary, testis, and brain. Use of “Omics” approach led to identification of new pathways and biological endpoints, and findings are relevant to better understanding of the mechanisms of health impacts in fish and other vertebrate species. The authors acknowledge funding from NSERC of Canada.

8 "Toxicant discovery in the zebrafish;" Using zebrafish to identify novel chemicals in environmental samples

J. Leèrdal, Institute for Environmental Studies; K. Ouyang, Institute for Environmental Studies IVM, P. Cenijn, VU University Amsterdam / Institute for Environmental Studies; K. Kobayashi, Silicone Industry Association of Japan; C. Pylatiuk, KIT Karlsruhe Institute of Technology / Institute for Applied Computer Science; M. Lamoree, VU University, Institute for Environmental Studies; J. Legler, VU University / Institute for Environmental Studies Even the most sophisticated chemical analysis methods are swamped by the number of hazardous compounds found in the environment. Therefore, it became necessary to develop screening methods that are able to detect compounds in a high throughput manner (HTM). To improve the sensitivity of the bioassay detection, and to take advantage of the full potential of the zebrafish, we have extended the standard ZFET with additional more sensitive endpoints. The assays are all working within an high throughput 96 well automated format suiting EDA demands. Besides monitoring for general phenotypic malformations, cardiovascular toxicity is analyzed. The combination of endpoints allow us to screen large numbers of samples for multiple mechanisms of toxicity. Another great advantage of zebrafish in combination with EDA is the identification of substances that induce effects that are not detectable in the standard assay. These new approaches were used to investigate the mechanisms by which CECs disrupt reproduction and development in fish. Metabolomics studies demonstrate significant dysregulation of amino acid, lipid, energy, carbohydrate, nucleotide and cofactor/vitamin metabolism. Exposure of fish to mixture of chemicals resulted in relatively unique pattern of metabolism distinct from individual contaminants in gonad, liver and brain. In separate experiments, exposure of fathead minnow to environmental contaminant mixtures resulted in significant changes in transcriptome profiles, using well-characterized microarrays. We identified candidate low-dose biomarkers of chemical exposure in fish, and in combination with metabolomics data, the results provide critical information on cellular response following exposure to CECs. In zebrafish, exposure of developing embryos to contaminants resulted in precocious neurogenesis via an androgen receptor and Aromatase-B mediated mechanism, linking neurodevelopmental effects with sex as a consequence of changes in timing of neuronal birth. The results of our studies can be used to assess risk through mechanism-based understanding of cell and tissue response. The overall results support the hypothesis that different chemicals present in the Oldman River disrupt fish health by altering gene expression profile and metabolism of the liver, ovary, testis, and brain. Use of “Omics” approach led to identification of new pathways and biological endpoints, and findings are relevant to better understanding of the mechanisms of health impacts in fish and other vertebrate species. The authors acknowledge funding from NSERC of Canada.

9 Characterization of a zebrafish model for acute organophosphates poisoning M.D. Faría, CESAM, University of Aveiro / Biology; E. Prats, CSIC; F. Padrós, FFDS Universitat Autònoma de Barcelona; P. Babin, Universités Bordeaux 1 et 2; J.-C. Capdevila, University Bordeaux II, N. Vire, Mississauga, Canada; A. Knoll-Gellida, G. Mathieu, A. Tinguada-Sequeira, MRGM-Université de Bordeaux; F. Le Bihanic, EPOC / Bat B e me etage: A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; D. Raldua, IDAEA-CSIC Organophosphorus (OP) compounds are currently the most commonly used pesticides in the world. Acute OP poisoning (OPP), with around 3 million cases and 300,000 deaths annually, is a major public health problem. The molecular initiating event leading to OPP is the inhibition of the acetylcholinesterase (AChE), resulting in the accumulation of the neurotransmitter acetylcholine (ACh) in the cholinergic synapses. However, the pathways conducting to some of the adverse outcomes in OPP, especially those related to the intermediate syndrome (IMS), are not...
well-understood. Zebrafish is an animal model increasingly used in biomedical research, including human toxicology as well as ecotoxicology. Although the modes of action (MoA) leading to toxicity for OP pesticides in zebrafish embryos has been recently described, reported adverse pathways are more related to the developmental toxicity of OP compounds than with the OPP. In this study we use a prototypic OP compound, chlorpyrifos-oxon (CPO), to develop a zebrafish model of OP toxicity. Our acute effects of mild, moderate and high CPO concentrations on zebrafish larval development at different levels of organization including molecular (transcriptomic analysis by RNAseq, biochemical responses), cellular, tissue and organisinal (gross morphology and behavioral effects [visual motor response (VMR) and touch-evoked escape response]). Three phenotypes were identified by gross-morphology and behavioral analysis. The mildest phenotype was a progressive decrease in the VMR paralleling the inhibition of the AChE activity. The second phenotype was characterized by a shortening of the trunk length and paralysis, what is consistent with the nicotinic symptoms of an acute cholinergic crisis, where the AChE inhibition results in a progressive accumulation of ACh in the neuromuscular junctions, overactivation of the nicotinic ACh receptors and overexcitation of the muscle fibers. Finally, the most severe phenotype, induced mainly by high CPO concentrations, was related to the induction of oxidative stress in the central nervous system and muscle fibers, with a strong disruption in both tissues. In conclusion, the adverse outcome pathways of the different phenotypes have been dissected empirically and the results obtained show that exposed zebrafish mimic many aspects of the human OPP, therefore allowing the use of this model for identifying new antidotes against cholinergic syndrome and IMS.

10 Zebrafish embryos as a model to assess oxidative stress in vivo M.M. Santos, Azti-Tecnalia / Food Quality Safety and Identity; N. Conlledo, A. Barranco, M. Pardo, S. Rainier, AZTI tecnical and high CPO concentrations, was related to the induction of oxidative stress in the central nervous system and muscle fibers, with a strong disruption in both tissues. In conclusion, the adverse outcome pathways of the different phenotypes have been dissected empirically and the results obtained show that exposed zebrafish mimic many aspects of the human OPP, therefore allowing the use of this model for identifying new antidotes against cholinergic syndrome and IMS.

11 Investigating the developmental toxicity and molecular mechanisms of thyroid disruption in brominated flame retardant-exposed zebrafish (Danio rerio) A. Parsons, University of Exeter / Biosciences; M. Shinihi, National Institute for Basic Biology; T. Iuchi, National Institute for Basic Biology / Molecular Endocrinology; T. Hutchison, Centre for Environment, Fisheries, and Aquaculture Sciences (CEFAS); T. Kudoh, University of Exeter; C.R. Tyler, Biosciences College of Life and Environmental Sciences

In fish thyroid hormones mediate various developmental processes, influencing brain and skeletal development, regulating the rate of early life development and playing a key role in the events of metamorphosis. Brominated flame retardants (BFRs) are found at high levels in the environment, primarily by altering thyroid hormone homeostasis. Exposures to BFRs has been shown to disrupt circulating levels of T3 and/or T4 in rodents and fish. The molecular mechanisms underlying BFR-induced developmental toxicity and thyroid disruption are, however, still poorly understood. Though many traditional BFRs (PBBs and HBCDs) have recently been phased out they are still widely detected in the environment. Furthermore, in order that existing fire safety regulations are met, the use of replacement BFR products (such as BTBPE and DBDPE) is increasing. These novel BFRs are similar in structure to their predecessors and are a growing concern given their potential to act as thyroid disruptors. We have developed a reporter gene assay for screening various BFRs for interactions with zebrafish thyroid receptors (TRα and TRβ). This allows us to compare the mechanistic pathways of traditional BFRs with the novel BFRs in vitro. Furthermore we have developed whole mount in situ hybridization (WISH) and qRT-PCR assays to establish the ontology of genes in the hypothalamic-pituitary-thyroid (HPT) axis of zebrafish embryo-larvae. We subsequently have investigated the induction/suppression of these genes upon such environmental exposure to the thyroid hormone, triiodothyronine (T3). We have found that exposure to the T3 induces increases in the expression of several thyroid related genes. For example, exposure to 30 and 100 μg/L T3 induced a 1.4 and 1.5 fold increase in deiodinase expression in 36 hours post fertilization (hpf) larvae, respectively. These data provide the fundamental underpinning for future studies to investigate the effects of BFR exposures on the thyroid axis in developing zebrafish. Keywords: thyroid, zebrafish, gene, flame retardant

12 A Temporal High-Resolution Investigation of the AhReceptor Cascade during Early Development of Zebrafish (Danio rerio) after PCB126 Exposure H.A. Alert, RWTH Aachen University / Institute for Environmental Research BioK, L. Lademann, H. Hollert, RWTH Aachen University / Institute for Environmental Research; S.H. Keiter, Oebro University / MTM Research centre

The fish embryo acute toxicity test (FET) has become a well established method for the evaluation of chemicals for regulatory purposes. In addition, several studies approved the relevance and transferability of the test results to adult fish so that the FET has become an OECD guideline in 2013 (OECD 230). In addition, the FET can be used to get a more comprehensive inside of the AhR mediated metabolism at the gene expression, but also on the protein level during early life-stages of the early development of zebrafish (Danio rerio). In the present study zebrafish embryos were used to investigate the arylhydrocarbon receptor (AhR) signalling pathway in very short time intervals of 4 h from 2 h after spawning until 120 hpf. The embryos were continuously exposed to PCB 126 which is well known to be a strong inducer of the AhR pathway and still induces within 24 hpf the expression of the cytochrome P450 AhR. Using quantitative real-time PCR we measured the expression of all genes involved in the signalling cascade of the AhR pathway. In addition, we quantified the activity of induced biotransformation enzymes under unexposed conditions as well as under the exposure of PCB12 after using a kinetic form of the EROD-assay. Together, we aimed to get a more comprehensive inside of the AhR mediated metabolism at the gene expression, but also on the protein level during early life-stages of zebrafish embryos.

13 Systems Biology: increasing the capacity, understanding ecosystems and response mechanisms to model chemicals (including nanomaterials) (Mechanistic understanding (Meta-Omics))

Careful with that shotgun Eugene: the challenge of inferring 18S and 16S diversity profiles is via the use of amplicon sequencing. While this approach is fast, relatively rapid and can capture a wide breadth of biota, artefacts associated with PCR hinder the technique’s ability to obtain reliable proportional abundance data. New metatranscriptional methods exist that make use of these artefacts but currently, the potential to obtain unbiased proportional abundance compositional data is a tantalizing thought for many molecular ecologists. However, before we jump into routinely using metagenomic data to make assumptions about ecological condition, it is pertinent to thoroughly understand the attributes associated with metagenomic compositional data. Using a k-mer based approach, we examined 18S and 16S compositional profiles from sixty metagenomes obtained from three estuaries. DNA was sequenced using 12 lanes of Illumina HiSeq2500 (150 PE), with each sample equating to 1/5th of a lane. To examine the effect of sample depth on 16S and 18S fragment richness, an additional lane was used to sequence a single sample. The sample sequenced on a whole lane produced more than 2.93 x 10^8 reads, with
14 Microbial and shotgun proteomics to unravel individual and ecosystem responses in a long-term exposure to propranolol

A. Campos, CITMAR; G. Danilenko, Stockholm University / Biochemistry and Biophysics; S. Cristobal, Linköping University / Department of CI and Exp Medicine Cell Biology

Pharmaceuticals are an important group of environmental contaminants. By combining a microbial exposure and shotgun proteomics approach, we could study the proteomic response of mussel species, M. edulis, to a long-term exposure to propranolol. The specific goals were: i) elucidate the main molecular pathways affected by the pharmaceutical, in the Baltic Sea blue mussel, and to perform a functional analysis to the physiological response of this organism and ii) to compare the individual species response with a population response from metaproteomics analysis of Baltic soil. A 4-6 weeks microcosm experiment was conducted to study the effects of propranolol on 1000 µg/l on a Baltic Sea sediment containing the macroalgae (Ceramium tenuicorne), amphipods, mussels (Mytilus edulis) and sediment. This study enabled to observe the interference of the pharmaceutical with the inter-specific interactions, and that mussels are the most sensitive species, showing poor condition for survival. Here we report a label-free shotgun proteomics to investigate the molecular events that underlie the physiological condition of mussels exposed to the pharmaceutical. Several mussel tissues were dissected and cytosolic, membrane and nuclei proteins fractionated by differential centrifugation. Proteins were analyzed by nano-LC-MS/MS. Protein identification was achieved by searching the MS/MS data against a customized protein sequence database which included sequences from a public repository (UniprotKB). The response to the taxonomic group Mollusca, and sequences derived from RNA-Seq data. A metric method, based on spectral counting, was considered to evaluate the quantitative variations in protein expression. From a total of 663 proteins identified within biological replicates, 143 proteins were differentially abundant (control vs propranolol exposed groups, t-test P < 0.05). Functional classification analysis highlighted the effects of propranolol in proteins with binding, catalytic activity and structural function. These proteins demonstrate a relation to the compound, and provide hypothesis regarding its mode of action in non-target organisms like marine mussels. By integrating data from individual species response to stressors with ecosystem response, we can explore the prediction capabilities of these methodologies and the links with adverse outcome pathways.

Keywords: shotgun proteomics, microcosm, propranolol, mussel

15 Metaproteomic analysis, next generation environmental assessment in aquatic environment

S. Cristobal, Linköping University / Department of CI and Exp Medicine Cell Biology; B. Mathew, Linköping University / Department of Clinical and Experimental Medicine Cell Biology; A. Soggiu, P. Roncada, University of Milan / Department of Veterinary Sciences and Public Health DIVET; J. Kuruvilla, Linköping University / Department of Clinical and Experimental Medicine Current metaproteomics techniques could provide a wide resolution of proteins from a complex ecosystem biological or environmental. The qualitative and quantitative differences in this process provides a general level of complexity: at the community level, how the communities respond to the exposure, stress or disease; at the molecular level, which protein families have been expressed; and at the biochemical level, which enzymatic reaction or macromolecular machines have acquired a relevant role to maintain the community in equilibrium. Thus, metaproteomics analysis could offer a molecular snapshot of the microorganisms community at a specific location or time. This alternative methodology has been applied to analyse the functional role of the microbial community in food quality, in bioremediation, characterizing how microbial species could remediate toxic metal contamination in soils and ground waters [1], in carbon cycling, defining the role that microbial species have in the flow of carbon in a given ecosystem [2], in bioenergy, by understanding how microbial species might help convert cellulosic material to biofuels [3] or most extensively in human health and personal medicine, identifying how microbial species impact/control disease vs health in organs [4, 5]. However, metaproteomic analysis has not yet explored to implement traditional environmental assessment. Environmental assessment has traditionally focussed on evaluating the environmental quality by measuring abiotic components. The fundamental goal of a metaproteomic environmental assessment is to deliver a robust evaluation the environmental impact at population, individual, cellular and molecular level from one single, comprehensive and cost-efficient methodology. As a probe of concept, we presented the metaproteomic analysis of Baltic Sea soil exposure to propranolol for 6 weeks in a microcosm and the effects of salinity changes have also been evaluated. We tested if a metaproteomic-based assessment can estimate: i) variation in the abundance of proteins (i.e.) changes in biodiversity and ii) changes in the equilibrium of a marine soil ecosystem after exposure at a stressor. Keywords: metaproteomics, microcosm, environmental assessment, sediment

16 Microbial Resources and Omics approaches in ecotoxicology and bioremediation of polluted sites

O. Sibourg, J. Monier, C. Baguelin, S. Entessangles, C. Malandain, ENOVOE Successful site remediation through microbial degradation requires a thorough understanding of the microbial mechanisms implicated in the biodegradation of the pollutant in question. Regardless of the nature of the pollutant or environmental matrix, the success of a bioremediation project hinges on the consideration of two essential components: the presence of a microbial community which can be stimulated in order to promote biodegradation activity and the bioavailability of the pollutant (often influenced by the chemical and physical characteristics of the site). Understanding microbial degradation processes is therefore a major contributor to the success of such projects. ENOVOE’s biotechnological solutions include the investigation of microbial diversity involved in the degradation of the activity of indigenous bacteria. On-going technological advancements in the fields of metagenomics, metatranscriptomics and metaproteomics continue to bring new perspectives to our understanding of microbial mechanisms. Our presentation will illustrate how different molecular biology tools can be utilized to characterize and manage microbial resources, from the development of the remedial action plan to site treatment and closure, to identify any potential difficulties associated with up-scaling, and to enable the optimization of depollution strategies.

17 Using Next-Generation Sequencing to explore the impact of Titanium dioxide nanoparticles on the functional microbial diversity involved in soil N cycling

M. Simonin, Microbial Ecology Lab / UMR; T. Pommier, Ecologie Microbienne INRA University of Lyon; A. Dubost, CNRS University Lyon I / Microbial Ecology Lab UMR; J.M. Martins, LTHE University of Grenoble; A. Richaume-Jolion, Microbial Ecology Lab UMR

Titanium dioxide nanoparticles (TiO2-NPs) are commonly used in diverse applications such as paints, cosmetics, or paper production, and there is a growing concern about their potential environmental impact. Understanding the diversity of N-cycling processes along their life cycle from production to disposal, TiO2-NPs can be released in the environment, especially the soil through various pathways, such as agricultural amendments of sewage sludge or landfills [6]. The environmental release of TiO2-NPs raises concerns about the potential effect on soil functioning [7]. The impact of this emerging pollutant on soil nitrogen cycling was assessed on the diversity and the activity of different microbial groups involved in the nitrogen cycle in a microcosm experiment. Here we investigated how the diversity-functioning relationships are modified under this environmental pressure. A grassland soil was exposed up to 90 days to an environmentally relevant concentration or to an accidental spiking of TiO2-NPs (1 and 500 mg kg-1 dry soil) in microcosms. Potential nitrification rates were measured and we used the Illumina sequencing platform (MiSeq) to explore the effects of TiO2-NPs on the diversity of bacterial and fungal communities in soil. Compared to control, NPs addition had no significant effect on bacterial diversity. However, TiO2-NPs increased the abundance of the Archaea domain, which is involved in the dissimilatory reduction of nitrate to nitrite. Furthermore, we found that TiO2-NPs induced a decrease in the richness of the bacterial community which can be stimulated in order to promote biodegradation activity and the bioavailability of the pollutant (often influenced by the chemical and physical characteristics of the site). Understanding microbial degradation processes is therefore a major contributor to the success of such projects.
Lorraine
Bactericidal effects of NP-TiO2 under dark condition are still the subject of debate. As an example, some authors found that NP-TiO2 have no effect on the laboratory model strain E. coli while others found for this strain EC50 values differing from 10 magnitude orders. Such contradictory results may be explained by multiple causal factors, including differences in the intrinsic characteristics of NP-TiO2 and bacterial cell membrane. Experiments in this study, exposure conditions for toxicity assessment and even the method used to assess the toxicity, the latter being often inappropriate for nanoparticle toxicity assessment. Increasing knowledge about exposition parameters which trigger NP toxicity and associated molecular mechanisms would contribute to properly evaluate and predict NP effects/late in the environment. In our study, analysis of the interactions between nanoparticles (NPs) and bacteria highlighted the paramount role played by interfacial electrostatic interactions (NP-cell vs. NP-NP interactions) in determining the extent of NP toxicity and the importance of physico-chemical parameters such as pH and ionic strength in controlling these interactions. In condition of interaction, we showed that nanoparticles causes E. coli membrane depolarization and loss of membrane integrity leading to higher cell permeability. A transcriptomic analysis highlighted that deregulated genes are involved in the response to osmotic stress, metabolism of various cell envelope components and the uptake/metabolism of other endogenous and exogenous compounds. In addition, a significant number of deregulated genes encode proteins localized in the membrane and periplasmic space. All in all, results indicate that the primary effect of NP-TiO2 is initiated at the cell envelope level (membrane depolarization, loss of integrity) triggering an osmotic stress response in bacteria. These results are supported by the observed massive leakage of intracellular K+/Mg2+ content concomitant with the entrance of extracellular Na+, and by the depletion of intracellular ATP level.

Prioritization and management of multiple stressors in river basins: effects of chemical contaminants and natural stressors (I)

Managing multiple stress for multiple benefits: Towards new scientific concepts, methods and tools in river basin management
S. Birk, University of Duisburg-Essen / Aquatic Ecology; D. Hering, Aquatic Ecology
Water resources globally are affected by a complex mixture of stressors resulting from a range of drivers, including urban and agricultural land use, hydropower generation and climate change. Understanding how stressors interfere and impact upon ecological status and ecosystem services is essential for developing effective River Basin Management Plans and shaping future environmental policy. This contribution details the nature of these problems for Europe’s water resources and the need to find solutions at a range of spatial scales. In terms of the latter, we describe the aims and approaches of the EU-funded project MARS (Managing Aquatic ecosystems and water Resources under multiple Stress) and the conceptual and analytical framework that it is adopting to provide this knowledge, understanding and tools needed to address multiple stressors, MARS is operating at three scales: At the water body scale, the mechanistic understanding of stressor interactions and their impact upon water resources, ecological status and ecosystem services will be examined through multi-factorial experiments and the analysis of long time-series. At the river basin scale, modelling and empirical approaches will be adopted to characterise relationships between multiple stressors and ecological responses, functions, services and water resources. The effects of future land use and land use change in situ studies in European river basins will be assessed. At the European scale, large-scale spatial analysis will be carried out to identify the relationships among stress intensity, ecological status and service provision, with a special focus on large transboundary rivers, lakes and fish. The project will support managers and policy makers in the practical implementation of the Water Framework Directive (WFD), of related legislation and of the Blueprint to Save and Restore Europe’s Water Resources by advising the 3rd River Basin Management Planning cycle, the revision of the WFD and by developing new tools for diagnosing and predicting multiple stressors.

Multi-scale evaluation of multi-stressed ecosystems: an overview of a physical x chemicalstressorstudy
S. Devin, LIEC - Université de Lorraine - CNRS / LIEC CNRS UMR; P. Bauda, LIEC UMR Université de Lorraine CNRS; J. Baudoin, French National Agency for Water and Aquatic Environments; H. Clivot, Université de Lorraine / Laboratoire Interdisciplinaire des Environnements Continentaux; C. Pagnot, Université de Lorraine; D. Guerdon, ENSIACET / UPS CNRS / University of Lorraine
The proliferation of industries in the 20th century has induced a strong increase in metals inputs in rivers which have led to large-scale degradation in freshwater communities. Despite increasing severity in environmental legislation and recent decrease of metal degradations, high concentrations of metals are still present in sediments. In addition to this permanent toxic pressure, the increasing need of water has led to river hydromorphology management including an excessive dams and weirs construction. This combination of chemical and hydromorphological stressors not only impaired local biota, but also downstream ecosystems insofar as toxicants can be transferred to other aquatic compartments. However, while those two pressions often co-occur, they are rarely considered together, and ecosystem health evaluation generally focus on either hydromorphological or chemical stress. We explored an extended battery of indicators, based upon macroinvertebrate and microbial communities, to assess modifications from the population’ ecological niche to the ecosystem function with the six dam representing a gradient of sediment contamination. Three French rivers, belonging to the two river catchment, with two run-of-river dams on each, were selected to examine macroinvertebrates and microbial communities associated with various levels of sediment contamination. Macroinvertebrates communities were studied by surber sampling in the 10 river stations, and by a combination of dredge and surber sampling in the six dams. Collected data were processed through shape, leaf litter decomposition and benthi fluxes at the water / sediment interface. Our study, by integrating several compartments, allow to (i) identify the ecological consequences of the interaction between hydromorphological and chemical stress in aquatic ecosystems, (ii) identify the metrics that are the most suitable to evidence either single or multiple stress and (iii) to link the biological responses to each other through a conceptual diagram.

Joint and specific responses of biofilm and invertebrates to multiple stressors in Mediterranean rivers
S. Birk, Universitat de Girona; D. Barcelo, IQOAB-CSIC / Institute for Environmental Assessment and Water Research; R. Batalla, UdL; N. De Castro, Departamento DEcologia; A. Ginebreda, CSIC - Spanish National Research Council / Department of Environmental Sciences; M. Petrovic, ICRA; Y. Pico, University of Valencia / Medicine Preventive; L. Ponsati, E. Tornés, Catalan Institute for Water Research ICRA; I. Muñoz, University of Barcelona / Ecology
Rivers are submitted to a variety of stressors, including nutrient and organic matter excess, physical disturbances, hydrological irregularities, and the presence of chronic concentrations of inorganic and organic pollutants. The potential interactions between these stressors difficult attribute the effects to the biota. In this study we analyze the impact of co-occurring stressors on biofilms and invertebrates in four Mediterranean river basins. Biofilms were more reactive to hydrological stress compared to invertebrates and chemical conditions and their combination had an obvious impact on biological diversities and abundances in the two biological groups. Functional biomarkers (catalase activity for invertebrates, photosynthetic activity in the biofilms) evidenced the specific effect of emerging contaminants in one or the other. The presence of organic pollutants and the excess of nutrients and water conductivity were equally associated to the assemblage distribution of biofilms and invertebrates in every studied basin.

Assessment of multiple stressors in 4 Iberian river basins
M. Kuzmanovic; A. Ginebreda, CSIC - Spanish National Research Council / Department of Environmental Sciences; M. Petrovic, ICRA; D. Barcelo, IQOAB-CSIC / Institute for Environmental Assessment and Water Research
The potential interactions of stressors in Mediterranean rivers are particularly sensitive to anthropogenic influence since they are subjected to periodic droughts, consequent flow reduction and the decrement of rivers natural dilution capacity. This natural phenomenon is expected to increase further in the future in the context of global climate change. In this study we assessed the possible stressors for each of four studied Iberian river basins (Llobregat, Ebro, Júcar and Guadalquivir) including chemical stressors as: nutrients, organic and metals pollution together with hydrological and geomorphological stressors such as flow change and dam density. The dataset was gathered in the context of the multidisciplinary project SCARCE. It included more than 200 emerging and classical pollutants both organic and metals, nutrients and biological data that were sampled at 77 sites along the 4 river basins in autumn of 2010 and 2011. Hydrological data were available from the water authorities. Chemical data were transformed into toxic units (TU) to evaluate ecotoxicological risk of the compounds by dividing the measured concentration of chemical with the corresponding toxicity threshold for algae, invertebrates and fish. The resulting risk was mapped in GIS i.e. chemical stressors as sum of TU at each sampling site, nutrients (as concentrations) and hydrological stress. The final multiple stress risk was visualised by overlapping the individual risk maps. Multivariate statistic analysis was performed to distinguish the sites with different stress patterns. The highest overall risk was mainly in the small tributaries with the comparatively low flow and surrounded either by agricultural lands or urban zones which contributed to high input of nutrients and toxicants. Sites in the lower part of Llobregat basin (close to Barcelona area) and several in the upper area of Ebro basin (Basque Country) were mainly grouped by the characteristic urban and industrial type of pollution including pharmaceuticals, personal care products and metals. Most of the sites in the downstream part of Júcar were affected by agricultural
influence including pesticide risk and nutrient input. The overall risk and different stressors were identified for the studied river basins which could be used to prioritize the management measures to be implemented in order to improve both the chemical and ecological status.

23 Environmental context influences invertebrate community responses to anthropogenic perturbations in temperate streams

F J Burdon, Eawag Swiss Federal Institute of Aquatic Science and Technology / Department of Aquatic Ecology; K Rasinen; J Jokela, Eawag Swiss federal Institute of Aquatic Science and Technology; R I Eggen, Eawag / Department of Environmental Toxicology; C Stamm, Department of Environmental Chemistry Human population growth and land use change are contributing to increased environmental pollution, thus threatening aquatic biodiversity and ecosystem services that benefit society. Assessing pollutant impacts on the ecological integrity of receiving habitats are often based on macroinvertebrates indicators, but there is little known about the environmental factors that influence community sensitivity to perturbations such as wastewater discharges, which can have detrimental effects on the health of aquatic ecosystems. We predicted that community sensitivity would be strongly contingent upon environmental context (e.g., catchment landuses) mediated through proximate effects on upstream assemblages, as opposed to the magnitude of disturbance (e.g., wastewater dilution factors and composition), but that trait-specific indices might be more sensitive. To test our hypotheses, we sampled macroinvertebrate communities in twelve Swiss streams, with the integration of ecological knowledge and behavioral changes, and assessed a variety of environmental factors at the local and catchment scale. We found that consistent with previous studies, wastewater discharges were associated with decreased alpha diversity and altered composition of macroinvertebrate communities downstream. Catchments with intensive landuses (e.g., cropping) had more pollution-tolerant assemblages, meaning that upstream community composition and water quality were the main determinants of total community sensitivity than the magnitude of the disturbance (e.g., wastewater discharges). In contrast, trait-specific indices were more sensitive to the effects of wastewater. In summary, wastewater influences on total invertebrate community sensitivity were context-dependent, but trait-based indices were able to discern the magnitude of wastewater disturbances against a background of environmental heterogeneity. Our findings suggest that influences at the catchment scale shape macroinvertebrate community structure, and therefore determine the stability of assemblages exposed to local disturbances such as wastewater discharges. This helps us to better appreciate the potential influences of multiple stressors in spatially heterogeneous landscapes.

24 The art and science of integrating scientific knowledge for managing multiple pressures on river basins

A Slob, TNO / Strategy Policy; I V Meerkerk, Erasmus University Rotterdam / Public Administration; T Geerdink, TNO / Strategy Policy

The integration of scientific knowledge and behavioral changes are necessary to face multiple pressures on river basins, requires an in-depth cooperation between many disciplines from social and natural sciences, like biologists, eco-toxicologists, hydrologists, geologists, economists, spatial/planner urban planners, sociologists, policy analysts, etc. However, interdisciplinary work is not self-executive due to epistemological differences between scientific disciplines and their different approaches. Every discipline has its specific field of expertise, ways of generating relevant and reliable knowledge. A fundamental challenge is therefore to overcome problems created by differences in disciplinary cultures: to cross the conceptual and methodological boundaries between the prevailing fields of research. We argue that integration of knowledge for river basin management requires interdisciplinary science. Interdisciplinary research is conceptualized with four dimensions: the scope or breadth of interdisciplinary research; the degree of interactive research and integration of research components; The degree of integrative understanding; Effectiveness in advancing understanding The four dimensions will be illustrated with the evaluation results of the interdisciplinary research of the FP7 ARCH-project that researches the multiple pressures on lagoons, estuaries and fjords. This project has a specific process and evaluation work package that shapes and monitors the progress of the interdisciplinary work. The following factors appeared to enhance interdisciplinary open mindedness of the project partners, trust building between the partners, the role of facilitative leadership, the importance of developing a common vocabulary and the role of boundary objects (like joint models, maps or reports). In the ARCH project these different factors are facilitated for the project managers and are structured in the process design of the ARCH project. Furthermore, the first results of the monitoring of the interdisciplinary work in the FP7 project GLOBAQUA will be presented. The results of the ARCH-project show the specific work package facilitated and enhanced the interdisciplinary research in the project. The monitoring of the interdisciplinary work gives feedback on the progress of the interdisciplinary work. The process design is supporting the interaction between the interdisciplinary work. This supports the creation of a joint vocabulary and joint learning.

Metal in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (I)

25 Correlating in Vitro Bioaccessibility to in Vivo Relative bioavailability for As and Pb in Housedust in China

I Ma, Nanjing University / School of Environment; H Li, J Li, Nanjing University; A Juhasz, University of South Australia / Centre for Environm. Risk Assessment & Remediation

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Indigestion of household dust is an important exposure pathway for As and Pb in children. However, compared to soils, assessment of As and Pb relative bioavailability (RBA) in housedust is limited. In this study, 24 housedust samples containing 4.48–38.2 mg kg \(^{-1}\) As and 25.0–738 mg kg \(^{-1}\) Pb were assessed for As and Pb bioaccessibility using the gastric (GP) and intestinal phase (IP) of the Solubility Bioaccessibility Research Consortium method (SBRC), in vitro gastrointestinal method (IVO), and physiologically based extraction test (PBET). The As and Pb RBA in 12 samples was 7.0–38 mg kg \(^{-1}\) As and 63–738 mg kg \(^{-1}\) Pb using a mouse blood model. Both As and Pb bioaccessibility differed significantly with in vitro assays. The SBRC with lower GP pH produced significantly higher As and Pb bioaccessibility due to enhanced As and Pb dissolution. Lead bioaccessibility values from the IP of all assay procedures were considerably lower than the GP possibly due to Pb co-precipitation with Fe and re-adorption onto dust matrix. However, decreased As bioaccessibility from GP to IP was only found for the SBRC and IVO. Arsenic RBA ranged from 21.8 to 85.6% with samples containing low Fe and high OC having lower As-RBA while Pb-RBA ranged from 29.1 to 60.1%. Strong in vivo–in vitro correlations (IVIC) were found between As-RBA and As bioaccessibility for SBRC and DIN \((r^2=0.63–0.85)\), but weaker correlations were observed for IVO and PBET \((r^2=0.29–0.55)\). Similarly, Pb bioaccessibility based on the gastric phase of SBRC and DIN was correlated with Pb-RBA with \(r^2=0.68\) and 0.85. Our data suggested that both SBRC and DIN had potential to assess As and Pb bioavailability in housedust samples. However, more research is needed to develop a valid in vitro method to predict As and Pb bioavailability in housedust.

26 A correlation between nickel/metal mineralogy and its bioaccessibility in artificial (OECD) spiked soils

L Vasilev, University of Guelph / School of Environmental Sciences; M Dutton, Vale Inco; A Amendola, Golder Associates Ltd.; L Van Loan, Canadian Liabilities Source Inc; B A Hale, University of Guelph / School of Environmental Sciences

In Ontario, Site Condition Standards for soils are based on the "total" metal concentration in soil and it is the exceedance of those that may prompt an ecological RA; therefore knowledge of a change and transformation of metals in sites that are exceeding these standards is critical. The effect of field-aging on nickel (Ni) transformation in soil was studied using artificial OECD soils spiked with four Ni forms (NiSO\(_4\), NiO, NiS, and NiS\(_2\)) and two Ni mixtures (NiO/NiS\(_2\) and NiS/NiS\(_2\)). The mineralogy of the Ni in artificial soils after aging was characterized using XANES and Zatka sequential leaching protocol, with the goal of explaining variation in Ni bioaccessibility among soils. Total Ni recovery after wet-dry cycles designed to artificially age the soils, was variable among different Ni forms and Ni was more readily leached from soils amended with NiSO\(_4\) - the more soluble of the Ni compound, the greater the Ni leached from the soil and the poorer the total Ni recovery. The XANES spectrum for the NiO amended soil indicated that the oxide remained unaffected after aging. In all other soil samples, the predominant Ni species were Ni\(^{2+}\) and NiO. The XANES indicated that aging resulted in an oxidation of the Ni present in the samples. Zatka leaching analysis of soils spiked with NiO alone was in a good agreement with the XANES results: NiO remained unchanged after aging. Zatka analysis showed that Ni\(^{2+}\) salts in the artificial soils were not soluble in the water, but soluable by ammonium citrate and most likely to be NiCO\(_3\). To evaluate the extent of bioaccessibility of Ni and its further correlation with mineralogical phase, two in vitro methods were used: Pb (gastric) and SBCR. Overall, Ni bioaccessibility assessed using SBCR was almost twice as high as Ni bioaccessibility using gastric PBET. Linear regression between bioaccessible Ni andionic Ni estimated by XANES had a better correlation with SBCR values, thus likely to be more useful in discerning differences among soils. Ni "accessibility" from soil is a function of speciation, particle structure, and dissolution kinetics. Bioaccessibility simply enables adjustment of exposure estimates in risk assessment. Speciation analysis sequential extraction provides the underlying scientific context supporting the use of bioaccessibility in risk assessment.

27 Modelling arsenic and lead bioavailability in Canadian background soils
28 Implementation of a new micro profiling and micro sampling system to investigate fraction related processes at the sediment water interface

A. Fabricius, Federal Institute of Hydrology / G aquatic chemistry; L. Dueter, D. Ecker, German Federal Institute of Hydrology; T. Ternes, Bundesanstalt für Gewässerkunde.

With aquatic systems, the exchange of substances between the sediment and the overlying water is mainly driven by the steep biogeochemical gradients at the sediment water interface (SWI). The rapidly changing conditions influence the availability of nutrients, organic or inorganic analytes. To gain a better understanding of the key factors and processes determining the fate of substances at the SWI, methods with a spatial high resolution are required. The toolbox to study these processes contains experiments using thin section and use of sensors and electrodes (e.g., O₂, redox potential, pH value). However, the methods available for pore water sampling often require the installation of the sampling devices at the sampling site and/or intensive preparation procedures that may influence the conditions at the area studied and/or the characteristics of the samples taken. Beside that, the time consuming analyses during the operation and fractionation of interest to address the processes that govern the transport and availability of compounds at the SWI. By setting up a novel micro profiling micro sampling system (“missy”) it was possible to implement for the first time a direct and autonomous sampling of small sample volumes (missy will be demonstrated by the results of exemplary experiments investigating trace metal/loid distributions along depth profiles of a freshwater sediment core. Moreover, questions concerning particle driven (transport) processes will be discussed in relation to the O₂ concentration, redox potential and pH value measured in parallel to the pore water sampling. Besides natural processes, the technique enables to investigate (potential) effects of anthropogenic disturbances (e.g., pollutants or mechanical disturbances). Due to an increased use of engineered nanoparticles (ENPs) in numerous products and results will be presented.

29 A comparison of two techniques for quantifying [Zn+]2+ in estuarine waters

H.B. Pearson, Plymouth University / SoGESS; J. Galceran, Universitat de Lleida / Departement de Quimica; E. Companies, Quimica; S. Comber, Plymouth University / Environmental Science; C.B. Braungardt, Plymouth University / School of Geography, Earth and Environmental Sciences; J. Puy, Chemistry; P. Worsfold, Plymouth University / Geography, Earth and Environmental Sciences.

Keywords: Zinc; metal speciation; estuaries; analytical Zinc (Zn) is an essential element for the healthy development of all organisms, but uptake in excess can be harmful. The free metal ion is known to be the most readily bioavailable and is therefore of highest concern with respect to toxicity. Zinc is now classified as a specific pollutant by the UK Environment Agency, and changes to Environmental Quality Standards (EQS) in 2013 under the Water Framework Directive (WFD) has resulted in a revision of the standard for saline waters from 612 nM dissolved zinc to 121 nM including an ambient background concentration of 17 nM. The limited data upon which this standard is based has driven further research into methods which may be used by regulators and researchers to investigate links between metal speciation and potential biological effects. The use of the Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) technique for directly determining [Zn+]2+ is well established for marine and low ionic strength waters, but has yet to be applied to estuarine waters, which are thought most at risk regarding compliance with new EQS. This research demonstrates, for the first time, the application of AGNES to estuarine waters from a Zn contaminated estuary in the SW of England. Data obtained from traditional complexity concentration titrations (CCT) with adsorptive cathodic stripping voltammetry (AdCSV) is compared to that directly obtained via AGNES. Water samples from the Tamar Estuary, UK have been collected over a full range of salinities (~0.1 – 35 psu) each season throughout a full calendar year. Samples were taken from locations suspected of being influenced by different ligand sources. Samples were filtered to 0.4 µm and 0.2 µm, and were analysed for DOC, nutrients, and chlorophyll-a concentrations. The total dissolved and labile Zn concentration, complexation capacity, natural ligand strengths (log Ks) and [Zn+]2+ have been measured. A critical comparison between the free Zn concentrations obtained from AdCSV and from AGNES has been conducted. The evolution of the different fractions of Zn (free, labile, non-labile) along the range of different salinities in the estuary is highly dependent on the season, which modifies the degree of estuarine mixing. Subsequently, seasonal variations in metal speciation should be considered when defining EQS.

30 Assessing the important silver binding ligands in surface and waste water: chloride, sulphide and dissolved organic matter

R. Nasir, Wilfrid Laurier University / Chemistry; S.D. Smith, Wilfrid Laurier University / Department of Chemistry; G. Merrington, Environnement Agency; A. Peters, Wca Environment Ltd.; S. Lofots, Centre for Ecology & Hydrology / Shore Section

Silver is of particular interest due to its use in consumer products as an antimicrobial agent, leading to concerns over its unintended release into the environment. The release of silver in natural water through waste water treatment plants, as well as direct inputs, could result in increasing free metal ion concentrations and potential toxicity. Therefore, understanding the behaviour of silver as it enters aquatic systems is critical to evaluating its potential environmental risk. Developing a complete understanding of interactions between silver and strong ligands such as dissolved organic matter (DOM) and thiol compounds present in water will allow for more accurate and robust predictions of silver speciation and hence bioavailability. This project aims to better understand silver complexation with dissolved organic matter (DOM), reduced sulphide, and chloride in both waste-water and freshwater systems. DOM samples were obtained from wastewater effluents as well as waste-water and treated river water systems. Two reference DOM samples were also obtained from International Humic Substances Society. DOM solutions of concentrations from 50-400 mg C/L were titrated with silver using a silver ion-selective electrode (ISE) in conjunction with an Ag/AgCl reference electrode. A pH of 6, or 8 (+/- 0.5) was maintained for all solutions. The titration data was then plotted and [Ag+] calculated using the Nernst Equation. Similarly, a cadmium ISE was used to determine Chloride [Cl-] concentrations obtained via AGNES. Water samples from the Tamar Estuary, UK have been collected for determination of metal speciation and potential biological effects. The use of the AdCSV technique for directly determining [Zn+]2+ is well established for marine and low ionic strength waters, but has yet to be applied to estuarine waters, which are thought most at risk regarding compliance with new EQS. This research demonstrates, for the first time, the application of AGNES to estuarine waters from a Zn contaminated estuary in the SW of England. Data obtained from traditional complexity concentration titrations (CCT) with adsorptive cathodic stripping voltammetry (AdCSV) is compared to that directly obtained via AGNES. Water samples from the Tamar Estuary, UK have been collected over a full range of salinities (~0.1 – 35 psu) each season throughout a full calendar year. Samples were taken from locations suspected of being influenced by different ligand sources. Samples were filtered to 0.4 µm and 0.2 µm, and were analysed for DOC, nutrients, and chlorophyll-a concentrations. The total dissolved and labile Zn concentration, complexation capacity, natural ligand strengths (log Ks) and [Zn+]2+ have been measured. A critical comparison between the free Zn concentrations obtained from AdCSV and from AGNES has been conducted. The evolution of the different fractions of Zn (free, labile, non-labile) along the range of different salinities in the estuary is highly dependent on the season, which modifies the degree of estuarine mixing. Subsequently, seasonal variations in metal speciation should be considered when defining EQS.

31 Can the urban fingerprint of silioxanes be characterised by their presence in different environmental matrices?

N. Ratola, LEPAE - University of Porto / Physics of the Earth; J.A. Silva, LEPAE University of Porto / V. Homem, University of Porto / LEPAEBDEQUEUP; A. Cincinelli, Lancaster University / Chemistry Dept; P. Jimenez-Guerrero, University of Murcia / Physics; L. Santos, Faculty of Engineering - University of Porto / Chemistry / Faculty of Engineering - University of Porto / Chemistry.

Siloxanes have recently been identified as emerging contaminants and are now raising concern among the scientific community, due to a wide use in personal care products and cosmetics and also in industrial applications. Having high vapour
pressure, volatilization to the atmosphere is the dominant mode of emission, but their presence was reported in different environmental media. Recent reports suggest toxic effects in several organisms, but there is a general lack of information about siloxanes, with not many monitoring studies available and even fewer modelling approaches. With an emphasis on the urban fingerprint they are prone to exhibit due to their anthropogenic sources and use, this study aims to improve the knowledge of levels, trends, and behavior rates of these organics in the environment. Adding to the knowledge in this area is the assessment of the concentrations in pine needles and soil was also performed, thus closing the circle of atmospheric exposure in the areas of study. Important in the initial concept of this work was the sampling strategy, comprising different locations, which allows to distinguish between a wide range of human presence, influencing the cycolic behaviour on siloxanes and the decisive influence of meteorology on atmospheric movements of pollutants. Acknowledgements: This work was funded by FEDER through the Operational Programme for Competitiveness Factors – COMPETE, ON.2-0 Nova Norte-North Portugal Regional Operational Programme and National Funds through FCT - Foundation for Science and Technology under the projects: PEst-C/EQB/UI0511, NORTE-01-0145-FEDER-020042, EXPL/AAG-MAA/0981/2013 and grant SFRH/BPD/76974/2011. Pedro Jimenez-Guerrero thanks the Ramon y Cajal programme.

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Biocides from façade coatings in urban surface waters: Estimating the leaching of biocides from render by polyacrylate-water partitioning constants? U. Bollmann, Aarhus University; K. Styszko, AGH University of Science and Technology; Dep Coal Chem Environ Science; Y. Ou, University Duisburg Essen; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; S. Trapp, Danmark Tekniske Universitet / DTU Environment; J. Vollenthin, Aalborg University; K. Bester, Aarhus University / Environmental Science

Leaching of biocides from façade coatings attracts more and more attention within recent years. In- and as well as film preserving biocides are added to polymer resin based renders and paints in order protect from microbial spoilage. However, several researches and campaigns (one in summer and one in winter), were done in a total of 8 points in 20 years, achieving an extensive characterization of soil uses. Given the novelty of this matter, the interpretation of results is essential and requires a comprehensive approach with the use of advanced statistical validation. At the same time, modelling approaches also have to be attempted, taking into account the chemical information available on siloxanes and the decisive influence of meteorology on atmospheric movements of pollutants. Acknowledgements: This work was funded by FEDER through the Operational Programme for Competitiveness Factors – COMPETE, ON.2-0 Nova Norte-North Portugal Regional Operational Programme and National Funds through FCT - Foundation for Science and Technology under the projects: PEst-C/EQB/UI0511, NORTE-01-0145-FEDER-020042, EXPL/AAG-MAA/0981/2013 and grant SFRH/BPD/76974/2011. Pedro Jimenez-Guerrero thanks the Ramon y Cajal programme.

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Release of benzothiazole and mercaptobenzothiazole from granules used for artificial turf


Synthetic rubber granules and turfs are increasingly used for the construction of outdoor sport grounds. For a safe usage not only the possible impact on human health but also the risk for the environment has to be investigated. The major pathway for transferring chemicals from granules to the environment is leaching by contact with rain water. Column leaching tests are often used for determination of the leachable amount as this cannot be predicted from total solid concentrations. In earlier studies mainly release of heavy metals and regulated organic compounds as PAHs have been investigated. The measured concentrations of these compounds were linked to toxicological effects. In toxicological studies of further organic compounds in the eluates revealed that mercaptobenzothiazole (MBT) is one of the major constituents. The aim of this study was thus to gain more information on leaching behavior of more polar compounds as MBT and benzothiazole (BT). Different rubber materials as styrene butadiene rubber, ethylene-propylene-diene monomer sulfur cross linked and styrenic thermoplastic polyurethane containing TBPH isomers were detected in dust (0.01%±0.67 OH\textsubscript{TBPH1} (0.04±0.88 ng/g) in dust, similar to the profile in urban wastewater and the atmosphere made up of scattered and heterogeneous sources of contamination should be considered.

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Detection, identification, and quantification of hydroxylated isomers of bis(2-ethylhexyl)-tetrabromophthalate

D.M. Saunders, University of Saskatchewan / Toxicology; h. peng, J. SUN, Toxicology Centre; G. Codling, University of Saskatchewan / Toxicology; S.B. Wiseman, University of Saskatchewan / Toxicology Centre; P.D. Jones, University of Saskatchewan / School of Environment and Sustainability; J.P. Giesy, University of Saskatchewan / Toxicology Center

Ultra-High Resolution LC/mass spectrometry (LC-UHRRMS; Thermo Fisher Q-Exactive) was used to identify two novel isomers of hydroxylated bis(2-ethylhexyl)-tetrabromophthalate (OH-TBPH) which were unexpectedly observed in a commercial standard of bis (2-ethylhexyl)-tetrabromophthalate (TBPH). By combining UHR mass spectra (MS1), UHR-MS2 spectra and NMR analysis, the structures of the two compounds were identified as hydroxylated TBPH with a hydroxyl group on the aromatic ring. Relatively great proportions of the two isomers of OH-TBPH were detected in two technical products, Firemaster\textregistered; 550 (FM-550) (0.1% and 6.2%, respectively) and Firemaster\textregistered; BZ 54 (BZ-54) (0.1% and 7.9%, respectively). To simultaneously analyze OH-TBPH isomers and TBPH in dust samples, a method based on LC-UHRRMS was developed to quantify OH-TBPH and TBPH using negative and positive ion modes, respectively. The instrumental limit of detection for TBPH was 0.01 µg/L, which was 200-300 times better than traditional methods (2.5 µg/L) based on gas chromatography-mass spectrometry. The analytical method contained with a Florisil clean up was successfully applied to analyze TBPH and OH-TBPH in 23 indoor dust samples from Saskatoon, SK, Canada. Two OH-TBPH isomers, OH-TBPH1 and OH-TBPH2, were detected in 52% and 91% of dust samples, respectively. Concentrations of OH-TBPH2 (0.35±1.0 ng/g) were 10-fold greater than those of OH-TBPH1 (0.04±0.88 ng/g) in dust, similar to the profile in FM-550 and BZ-54. TBPH was also detected in 100% of samples of dust with a mean concentration of 733±0.87 ng/g. A significant (p < 0.001) log-linear relationship was observed between TBPH and OH-TBPH isomers further supporting the hypothesis of a common source of emission. Relatively small proportions of OH-TBPH isomers were detected in dust (0.01%±0.67 OH-TBPH1 and 0.1%±0.60 OH-TBPH2), both of which were significantly lesser than those in
technical products (p < 0.001). This result indicated different environmental behaviors of OH-TBP and TBPH. Detection of OH-TBP isomers in this study is important, since compounds with phenolic groups have often shown relatively greater toxicities than non-phenolylated analogues. Further study is warranted to clarify the environmental behaviors and potential toxicities of OH-TBP isomers.

36 WASTEEFFECT: Life cycle effects of emerging contaminants in waste N.A. Morin, Environmental Engineering; S.E. Hale, Norwegian Geotechnical Institute / Environmental Engineering; G. Okkenhaug, Norwegian Geotechnical Institute; M.M. Sparrevik; P.L. Andersson, Umea University / Chemistry Department; A.R. Alnas, Norwegian University of Life Science; K. Breivik, Norwegian Inst. for Air Research; F. Wanja, University of Toronto at Scarborough / Department of Physical and Environmental Sciences; G.D. Breedveld, Norwegian Geotechnical Institute / Department of Environmental Engineering; H.H. Arp, NGI / Environmental Engineering

Waste treatment has become a dynamic industrial sector, which in many countries is changing from traditional landfilling to recycling and incineration approaches that help to almost obtain new and valuable materials and energy recovery, waste also contains a large variety of emerging contaminants, however how landfilling, incineration, and recycling can effect environmental emissions and exposures of such emerging contaminants remains largely unexplored. WASTEEFFECT is a research project funded by the Norwegian Research Council where the main goal is to develop robust waste emission and exposure models for waste regulators and companies to anticipate and reduce risks from emerging contaminants. Therefore, one of the main part of this project is to determine how emerging contaminants behave in different waste streams, including landfilling, incineration and recycling. For priority contaminants used in consumer products, waste treatment will play a large role in the ultimate removal of these substances from the environment. We have established the case study for a Norwegian waste emission inventory of legacy and new brominated flame retardants (BFRs), bisphenol A and antimony, in EE-waste, car waste, plastics, glass and combustible waste. Input parameters for these inventories have been obtained by sampling and analysing air, leachate water and solid samples from various waste treatment methods (incineration, recycling and landfilling), by considering the entire waste-life cycle. Results for bisphenol A show that a large subfraction is removed from the Norwegian environment through thermal decomposition (60%). Another subfraction is recycled (36%), and thus, not eliminated from the system. A small subfraction is “stored” in the system via landfilling (4%), though up to 20% of this deposited fraction can be introduced to the environment as water emissions or as sewage sludge used in agriculture. These data also allow us to establish the contribution of the waste sector to the net burden or exposure to the environment and humans. The main output of this research will help guide the waste management sector on which of the treatment methods provide the lowest risk in regards to emerging chemicals, and which processes could be optimized.

POPs and Pseudo-POPs: Environmental occurrence and exposure (I)

37 The Three Faces of P: Refocusing PBT on Exposure R.K. Boethling, Exposure Assessment Branch US EPA Office of Pollution Prevention and Toxics

Persistence has been defined as the ability of a chemical substance to remain in an environment in an unchanged form. The longer a chemical persists, the higher the potential for human or environmental exposure to it. Current assessment paradigms for Persistent, Bioaccumulative and Toxic (PBT) chemicals are the descendants of concerns about exposure to chlorinated pesticides (popularized in Silent Spring) and polychlorinated biphenyls (PCBs). “Pseudo persistence” occurs when the rate of input of a chemical exceeds rates of degradation and/or physical movement out of a given area. More important is the outcome, which is that a substance can accumulate to the point of being detectable in monitoring and/or having effects even if it is not especially resistant to molecular degradation. Here we place this in the context of a broader view of persistence, and use the flame retardant Firemaster 550 as a case study. This broader view includes molecular persistence, situational persistence and pseudo persistence, which can be called the Three Faces of P. In so doing we refocus attention on exposure assessment as the proper framework. “PBT” has come to be overused, and PBT criteria applied seemingly without thinking, because exposure assessment is difficult and PBT is simple. But this emphasizes simplistic and inaccurate assessment. Exposure assessment is the “more holistic” PBT paradigm that some have suggested is needed to account for the fact that substances that don’t meet P half-life criteria are increasingly found in monitoring. Further, progress can be made if we expand use of models to complement other information such as half-lives. Regulators must limit “PBT” as a substitute for exposure assessment, and must be ready to endorse conclusions based in part on modeling.

38 N-oxides - Emerging Transformation Products of Amine-containing Organic Micropolutants R. Gulde, Eawag Swiss Federal Institute of Aquatic Science / Environmental Chemistry; U. Meier, Eawag Swiss federal Institute of Aquatic Science; M. Ruff, H. Singer, Eawag Aquatic Research; L. Fenner, SETAC North America

Aliphatic amine functional groups are abundant in many pharmaceuticals and consequently chemicals containing aliphatic amines represent a significant fraction of emerging contaminants (EMCs). Data on their fate in wastewater treatment and whether any transformation products (TPs) are formed that might be released to surface waters. It is therefore of interest to examine to what extent aliphatic amines are biotransformed during wastewater treatment and whether any transformation products (TPs) are formed that might be released to surface waters. We carried out biotransformation experiments with structurally diverse amine-containing compounds in activated sludge-seeded batch reactors. Samples were analyzed with a liquid chromatography high-resolution mass spectrometer and suspect and non-target screening was used to identify TPs. Aliphatic amines were found to undergo multiple biotransformation reactions, but all examined tertiary aliphatic amines formed at least an N-oxide TP. Separate batch experiments with five N-oxides further showed their biotransformation was considerably slower than that of the respective parent amines, suggesting that the formation of N-oxides during wastewater treatment could lead to significant environmental exposure to these compounds. However, hardly any information on their environmental fate or toxicity could be found. We therefore screened the effluent of a full-scale wastewater treatment plant (WWTP) for 30 wastewater-relevant MPs that contain a tertiary aliphatic amine moiety and found qualitative evidence for N-oxide formation for 10 compounds. Reference standards were purchased for seven of these N-oxides and their effluent concentrations will be determined for 10 Summer and 4 German WWTPs. We further looked for N-oxide TPs in 11 samples along the river Rhine, that had been taken from the same parcel of water. The analytical data indicated occurrence of the N-oxide TPs of lidocaine and tramadol, and the pattern of their peak areas along the different sampling locations suggested that both the parent compound and the N-oxide TP show considerable stability in the river Rhine. The concentrations of N-oxide TPs in these samples will be quantified and correlate the stability of the N-oxide TPs more accurately. Overall, the occurrence of N-oxide TPs in WWTP effluents and river waters points towards environmental (pseudo-)presence of these compounds. Therefore, it will be important to also evaluate the potential for ecotoxicological effects of N-oxide TPs to fully assess their environmental risk.

39 BROMO- AND CHLOROCARBAZOLES IN SOIL - ARE THEY POTENTALLY PERSISTENT ORGANIC POLLUTANTS? J. Mumbo, Helmholtz Zentrum Muenchen / Molecular EXplosomics; B. Henkelmann, Helmholtz Zentrum Muenchen / G. Pflister, HMGU-MEX; K. Meier, Helmholtz Zentrum Muenchen / M. Eberhardt, J. Li, University of California, Riverside / Department of Environmental Sciences; J. Gan

Carbazole, 3-chloro- and 3,6-dichlorocarbazole were detected including trichlorocarbazole not reported previously in soils. The order of concentration beginning with the highest was, 3,6-dichlorocarbazole > 3-chlorocarbazole > carbazole > trichlorocarbazole. 3,6-dichlorocarbazole recorded consistently approximately 10 times higher concentrations in comparison to carbazole and 3-chlorocarbazoles during the study period. Carbazole and 3-chlorocarbazole showed significant dissipation at 15°C but not at 20°C incubating conditions indicating that low temperature could be suitable for dissipation of carbazole and 3-chlorocarbazoles. 3,6-Dichlorocarbazole was resistant at both conditions. Trichlorocarbazole however exhibited a tendency to increase in concentration with time. Pure compounds of 3-chlorocarbazole, 3,6-dibromocarbazole, and selected soil extracts exhibited enhanced toxicity. Dioxin-like toxicities were closely correlated significantly with time whereas the sum chlorocarbazole toxic equivalence concentrations did not contribute significantly to the soil assay dioxin-like toxicity equivalent concentrations. It is evident from this study that halogenated carbazoles are persistent compounds. A persistence of > 120 days recorded for carbazole and 3-chlorocarbazoles during the study period. Carbazole and 3,6-dichlorocarbazole were identified as a potential Biotransformation products containing Organic Pollutants. Separate batch experiments with five N-oxide TPs in WWTP effluents and river waters points towards environmental (pseudo-)presence of these compounds. Therefore, it will be important to also evaluate the potential for ecotoxicological effects of N-oxide TPs to fully assess their environmental risk.

40 Carbamazepine: A holistic look at its environmental persistence and fate J. Gan, University of California, Riverside / Department of Environmental Science; J. Li, University of California, Riverside / Department of Environmental Sciences; J.L. Conkle, Texas A&M University Corpus Christi / Physical and Environmental Sciences; X. Wu, University of California Riverside / Environmental Sciences; L. Dobbs, University of California Riverside / Environmental Sciences; N.A. Morin, Eawag Swiss Federal Institute of Aquatic Science / Environmental Sciencees; X. Wu, University of California Riverside / Environmental Sciences; L. Dobbs, University of California Riverside / Environmental Sciences; N.A. Morin, Eawag Swiss Federal Institute of Aquatic Science / Environmental Sciencees

The antiepileptic drug carbamazepine is one of the most frequently detected human pharmaceuticals in wastewater effluents and biosolids. Recent studies from this and several other groups have considered the persistence and fate of carbamazepine in soil, and its uptake and metabolism by plants. In soil, carbamazepine consistently exhibits longer persistence than many other PPCPs. For example, under aerobic conditions, the half-lives of carbamazepine in soil ranged from 1.5 to >6 months. Using 14C labeling, it was shown that mineralization was negligible and carbamazepine existed largely as the parent compound in the extractable form, suggesting great mobility and bioavailability. Under hydroponic and field environments, however, the persistence of carbamazepine is much lower, exhibiting halflives in the range of days to weeks.
Aquatic Nanotoxicology: from Freshwater to Seawater - overcoming the difficulties of standard toxicity tests and the implications for higher tier toxicity testing (I)

43 Toxicity of selected nano-objects and their aggregates and agglomerates (NOAA) to freshwater invertebrates

V. Ricottone, Heriot-Watt University / School of Life Science; T.B. Henry, Heriot-Watt University / School of Life Science; A.O. De Silva, Environment Canada; S. Smyth, Environment Canada / Water Science Technology Directorate; T. Peart, Eawag – Swiss Federal Institute of Aquatic Science and Technology; Z. Jaroń, Polish Academy of Sciences; E. Hite, Environment Canada; R. C. Buck, E. duPont de Nemours & Co., Inc.; G. R. Skar, Chalmers University of Technology; S. Malcherek, University of Gdansk; C. Bertrand, Université de Lorraine, CNRS UMR 7360; A. O. De Silva, Environment Canada; A. Oliveira, Universidade Federal do Rio Grande do Sul; C. Bertrand, Université de Lorraine; A.O. De Silva, Environment Canada; W. Langer, Université de Lorraine; and J. A. Zalouk, Université de Lorraine.

This research project is funded by the European FP7 project SUN "Sustainable Nanotechnologies" which aims to address the entire lifecycle of nano-objects and their aggregates and agglomerates (NOAA) (from cradle to grave) creating an integrated approach for the long-term sustainability of nanotechnologies through the development of a method to evaluate the potential release of nanomaterials and products, as well as methods reducing both adverse effects and exposure to acceptable levels.

Aquatic exposure studies were conducted on two model species: the cladoceran Daphnia magna and the snail Lymnaea stagnalis.

The experiments have started assessing the ecotoxicity on the aquatic ecosystem of prioritized pristine engineered NOAA (Org. Pig.-Red254, WC-Co, MWCNTs and CuO) and the different SUN nano materials. The initial studies were focused on the evaluation of the acute lethal toxicity of copper oxide NPs to juveniles (7-9 day old) of the pond snail L. stagnalis exposed for 96h at 20°C to Cu, either in the form of CuO NPs or as aqueous salt, CuSO₄·5H₂O. LC50 values were estimated, respectively, 2500 μg/L Cu and 5 μg/L Cu, indicating a greater toxicity on L. stagnalis of CuSO₄ than CuO NPs. Screening tests were conducted for the remaining prioritized nanomaterials using the crustacean D. magna, in order to assess the threat these NOAA might pose onto the ecosystem before proceeding with further investigations.

Overall results indicate a moderate toxicity for Org. Pig.-Red254 and a much lower toxicity for the other two NOAA, WC-Co NPs, and MWCNTs. Respectively, L/E/C50 immobilization values on D. magna for Org. Pig.-Red254, WC-Co NPs are: 1000 μg/L Org. Pig.-Red 254, 3370μg/L WC-Co NPs. For MWCNTs, in the range of exposure concentrations was only possible to detect the L/E/C20 with an estimation of 9600 μg/L. Long-term reproduction tests assessing the impact that these NOAA, their fragmented products (FP) and the weathered fragments products (WFP) may pose, based on Predicted Environment Concentration (PEC) on L. stagnalis, are ongoing. Endpoints, such as hatching rate, vitellogenin-like protein and quality of eggs produced by exposed snails will be evaluated.

44 Influence of salinity on fate and behavior of silver-based manufactured nanomaterials and toxicity effects toward two endobenthic bivalves Corbicula fluminea and Sphaeriodosiphus plana.

C. Bertrand, Université de Lorraine, CNRS UMR 7360; A. O. De Silva, Environment Canada; A. Chatel, Université de Lorraine, CNRS UMR 7360; S. Auffan, iCEINT / International Consortium for the Environmental Implications of Nanotechnology; M. Tella, CEREGE; C. Pagnout, Université de Lorraine; S. Devin, LIEC - Université de Lorraine - CNRS / LIEC CNRS UMR; L. Giamberti, Université de Lorraine, CNRS UMR 7360 / LIEC CNRS UMR; V. Stone, Heriot Watt University; T.B. Henry, Heriot-Watt University / School of Life Science.

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feeding condition had no significant impact on S.plana burrowing. For C.fluminerea, a significant increase of the burrowing kinetics was observed between the unfed and the fed conditions.

45 Bioaccumulation of silver and genotoxic effects in mussels Mytilus galloprovincialis exposed to PVP/PEI-coated silver nanoparticles through the diet at different seasons

N. Duróudier, A. Katsamti, University of the Basque Country UPV/EHU; J. Jimeno-Puerto, University of the Basque country UPV/EHU / Dept Zoology and Cell Biology / Research Centre for Experimental Marine Biology and Biotechnology PIEUPVEHU; M. Mikolaycz, J. Schafer, Université Bordeaux / UPR ATREM/CL : University of the Basque country (UPV/EHU) / Dept Zoology and Cell Biology; M. Cajaraville, University of the Basque country UPV/EHU / Zoology and Animal Cell Biology / Research Centre for Experimental Marine Biology and Biotechnology PIEUPVEHU

Keywords: silver nanoparticles, dietary exposure, Mytilus galloprovincialis, genotoxicity In the last years, silver nanoparticles (Ag NPs) have gained high commercial interest basically due to their antimicrobial properties. Applications for Ag NPs are increasing and thus, concerns about their potential input in aquatic ecosystems and their environmental hazards are also growing. Studies based on waterborne exposure have been reported, but there is a gap of knowledge on the potential toxic effects of Ag NPs ingested and transferred through the food web, especially at environmentally relevant concentrations. Further, the possibility that effects may differ depending on the season has not been explored. Therefore, dietary exposure experiments were performed with mussels Mytilus galloprovincialis, both in autumn and in spring, and bioaccumulation and genotoxic effects (based on Comet assay and micronuclei frequency) caused by Ag NPs were assessed at 1, 7 and 21 days. Mussels were fed daily with the microalgae Isochrysis galbana previously exposed for 24 hours to two different doses of PVP/PEI coated 5 nm Ag NPs; and a high dose of 10 µg/L Ag NPs in (spring) and to 10 µg/L Ag NPs (in autumn). Chemical analysis showed that microalgae exposed to 10 µg/L Ag NPs significantly accumulated Ag after 24 hours. Transmission Electron Microscopy observations evidenced electron dense deposits between the scales and the membrane of microalgae and inside cells, indicating internalization of Ag NPs in algae. Mussels fed with exposed microalgae significantly accumulated Ag after 7 and 21 days in both seasons. According to the Comet assay, both concentrations significantly damaged hemocytes DNA at 1, 7 and 21 days in spring. Micronuclei frequency showed an increasing trend after 1 and 7 days of exposure to 1 µg/L Ag NPs in spring and to 10 µg/L Ag NPs in both seasons, while after 21 days values returned to initial levels. Silver can be bioaccumulated by lower trophic level organisms and, then, incorporated into the food chain, reaching higher level predators. Thus, in this study we have especially focused in these two aspects: (1) to test the biological effects of an environmentally relevant concentration of Ag NPs in parallel with a likely effective dose, and (2) to test bioaccumulation and effects of silver transferred by the diet. For this, the brine shrimp (Artemia sp.) and the vertebrate model zebrafish (Danio rerio) were used to two different feeding conditions. First, the acute toxicity and bioaccumulation of PVP/PEI-coated Ag NPs of 5 nm was tested in both species. Then, Artemia larvae exposed to Ag NPs (0.1 and 100 µg/L) were used to feed zebrafish for 21 days. To study the fate and effect of silver in fish, autametallography, lysosomal membrane stability (LMS) test and histopathological analyses were applied. Ag NPs were much more toxic for zebrafish embryos (LC50 at 20h was 50 µg/L) than for Artemia larvae (LC50 ranging from 7.39 to 19.63 mg/L depending on the exposure conditions). At the two exposure concentrations, silver bioaccumulation was detected by autometallography in the intestine and liver of fish fed with contaminated Artemia and this silver transfer was able to impair fish health as reflected by the reduction of the LMS. In conclusion, in addition to being bioaccumulated and transferred through the food web, silver is able to exert toxic effects on organisms in a dose-dependent way, even at an environmentally relevant concentration, which should act as concern of the need of studies in further detail about real impact of nanomaterials in the environment and the need of usage regulations. Funded by Spanish Government (MAT2012-39372), Basque Government (IT620-13, IT810-13, Saiotek S-PE13UN142) and UPV/EHU (UI 11/37 and 11/52). The authors thank for technical and human support provided by SGiker of UPV/EHU.

47 Long-term exposure and recovery of Lemma minor to silver nanoparticles: the size matters

S.P. Pereira, University of Aveiro / Depart Biologia Universidade de Aveiro; F. Jesus, University of Aveiro / CESAM / CESAM Department of Biology; S.C. Aguiar, University of Aveiro / Department of Biology; R. Oliveira, University of Brasilia / Department of Genetics and Morphology; M.A. Fernandes, CESAM / Department of Biology / department of Biology & CESAM; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; A.J. Nogueira, University of Aveiro / Department of Biology symptome

The production of silver nanoparticles (AgNPs) increased exponentially in recent years, mostly due to their antibacterial properties that lead to a ubiquitous incorporation in consumer goods. This raises concerns about their environmental impact especially in aquatic systems. The AgNP’s toxicity to aquatic organisms depends on several factors, including the size: smaller AgNPs are often more toxic than larger AgNPs. Scarcity of studies about the effects of different AgNPs on aquatic plants remarks the need to understand how primary producers are affected. Lemma sp. standard toxicity tests run along 7 days (d); thus, long-term effects (individual and sub-individual levels) of AgNPs to Lemma minor remain largely unknown: longer availability might occur in real environmental conditions. This study aims to answer the following questions: Which are the long-term effects of AgNPs to L. minor? Do AgNPs differ in an individual and sub-individual level? How depend on AgNPs’s size? Do individual effects depend on exposure length? Do NP’s size affect L. minor recovery after long-term exposure? Hence, we exposed (28d) L. minor to two sizes of citrate-coated AgNPs (0.02-2 mg/L) – 10 nm (10-citrate) and 80 nm (80-citrate) – in Steinberg medium (SM) and then, allowed to recover (28d) in clean SM. We assessed the effects at the individual level – relative growth rate (RGR), determined each 7d, fresh weight and phenotypic characteristics – and at the sub-individual level – the enzymatic activities of catalase (CAT), guaiacol-peroxidase (G-Pox) and glutathione-S-transfere (GST). Both AgNPs-SM solutions had dispersed NPs or small agglomerates. 10-citrate affected more L. minor than 80-citrate, in both exposure periods. The individual endpoints grew suppression and the phenotypic effects – were more pronounced at higher concentrations of AgNPs, following a dose-response curve; however, sub-individual endpoints were more sensitive. Plants survived to recover when exposed to low concentrations and failed when exposed to high concentrations. These effects indicate that long-term exposure to AgNPs induced the production of ROS affecting strongly the plants and inducing damage. Long-term exposure even small differences between the two sizes of citrate-coated AgNPs observed after 7d of exposure. Further, ecotoxicological evaluations should be conducted for periods longer than 7d – OECD guideline N221.

48 Behaviour of TiO2 nanoparticles and bulk and its toxicity in freshwater and seawater microalgae

M. Sendra, I. Moreno-Garrido, CSIC Spanish National Research Council; J. Blasco, Inst. Ciencias Marinas de Andalucia / Instituto de Ciencias Marinas de Andalucia

The production of manufactured nanomaterials has risen exponentially in recent years. Despite this growth, information regarding the fate and behavior of nanomaterials (NPs) in freshwater and marine aquatic ecosystems is still limited. Whiteness, opacity and being a potent photocatalyst are just some of the features that make TiO2 perfect for a wide range of consumer applications such as paints, coatings, plastics, papers, printing inks, roofing granules, food, medicine, toothpaste, cosmetics and skin-care products (including topical sunscreens). Studies suggest that these properties are transferred to microalgae and the resulting products are more photocatalytic than rutile. However, the mixture of both is the most of the photocatalytic form of them. In this research, on one hand we have used anatase/rutile NPs with particle size of ≤100 nm, while on the other hand we have used conventional or bulk TiO2. The aim of the current study was to assess the toxicity of TiO2 in an aequous solution. Two aspects were studied under laboratory conditions, 1) NPs and Bulk TiO2 behavior with and without microalgae and 2) quantification of intracellular and extracellular NPs and Bulk TiO2. The experiment was performed for two types of microalgae Phaeodactylum tricornutum and Chlamydomonas reinhardtii, seawater and freshwater respectively. Both studies were carried out under light (UV-illumination) and white light. As regards TiO2 behaviour, aggregation was accelerated by UV irradiation and ionic strength due to salinity of sea water. UV irradiation is the cause of the formation of hydroxyl radical groups, which change surface charge and aggregation. Moreover, the presence of algae cells affects the stability of TiO2 suspensions. Both, nano and bulk TiO2 formed aggregates during incubation, but NPs TiO2 formed large aggregates trapped almost completely between algae more than bulk TiO2 did. The dispersion
of NPs in seawater is likely to be more difficult than in freshwater. It is reasonable to assume that the mechanisms of toxicity of NPs on marine organisms are more complex. Furthermore, the trend of quantification of intracellular and extracellular TiO$_2$ was similar. Within 72 hours of interaction period under UV and white light conditions, a greater proportion of NPs TiO$_2$ was found in the supernatant compared to the surface and interior of the cell. However, the highest concentration of extracellular and intracellular TiO$_2$ showed up with NPs TiO$_2$ under UV conditions.

Modelling of pesticides and biocides fate and exposure in a regulatory context (I)

49 The response of EFSA to the public consultation of the draft guidance document for predicting environmental concentrations (PECs) in soil A. Tiktak, Water Agriculture and Food; J. Boesten, Alterra / ERA team; M. Egsmose, EFSA; S. Karlsson, Swedish Chemicals Agency KEMI; M. Klein, Fraunhofer IME; M. Stemmer, Austrian Agency for Health and Food Safety / Institute for Plant Protection Products. In July 2014, EFSA launched an open consultation on the draft guidance document for predicting environmental concentrations of plant protection products (PPPs) in soil. This EFSA Guidance Document is intended to be used by applicants and authorities in the context of the review of PPPs under Regulation (EC) 1107/2009. Guidance is provided for all types of concentrations of active substances and metabolites of these substances that are potentially needed for assessing ecotoxicological effects to soil organisms, i.e. the concentration in total soil and the concentration in pore water. The procedure consists of five tiers; the first three tiers are described in the guidance document. User-friendly software was developed to facilitate consistent and efficient use of the tiered approach in regulatory practice. EFSA received some 300 comments from regulators, industry, research institutes and consultants. This has resulted in an update of the guidance document and will result in an update of the software tools. In this presentation, we will briefly summarise the Guidance Document and then discuss the most important changes that have been made in response to stakeholder’s comments.

50 The new TOXSWA 4.4.2: Impact within the regulatory context of pesticide authorization in the EU W. Reihler, A new version of the FOCUS surface water model TOXSWA 4.4.2 – used within the regulatory process of pesticide authorization in Europe – addresses formation of metabolites in water and sediment. As a consequence the lower tier STEP 1-2 tool was adapted as well providing predicted environmental concentrations (PEC) of aquatic metabolites to be in a reasonable order (Step 1 > Step 2 > Step 3). At Step 3 of the FOCUS surface water assessment, PECs are in principle calculated for the three different water bodies ditches, streams and ponds. Since for streams parent input from upstream catchment is taken into account within the FOCUS scenario setup a procedure addressing aquatic formation of metabolites in the upstream catchment was developed as well. Therefore, so-called correction factors were introduced, taking into account transformation rate of parent and metabolite referenced to average temperature of the water layer, formation fraction of metabolite as well as conservative assumptions on the average residence time in upstream catchment, i.e. the time available for degradation of a metabolite formed in the upstream catchment. Extensive simulations were calculated with old and new FOCUS sw tools (STEP 1-2, TOXSWA) considering different parent compounds combined with nine different primary metabolites each for all drainage and runoff scenarios parameterized for four FOCUS crops in total. Results show that the new approach may result in considerably higher new PECsw global max values for aquatic metabolites compared to a workaround described in the FOCUS surface water guidance from 2001 which also leads to higher base concentrations and increased time weighted average concentrations of aquatic metabolites. It is expected that in future Step 3 calculations for aquatic metabolites are required more frequently. The implemented procedure on addressing aquatic formation of metabolites in upstream catchment may lead to over-conservative high concentrations; the conservative nature of this approach is useful for representing a realistic worst-case-interest at the same time the simplifications of the approach demonstrate that further refinement option should be developed. Some potential refinement options will be stated.

51 GERDA - Geo-based [German] Runoff, erosion and Drainage risk Assessment D. Grossmann, M. Bach, University of Giessen; M. Diesner, German Federal Environment Agency UBA; D. Guerniche, RLP AgroScience GmbH; U. Hommen, M. Klein, Fraunhofer IME; R. Kubia, RLP AgroScience; A. Mueller, Federal Environment Agency / IV; J. Priegnitz, Umweltbundesamt / FG IV Pflanzenschutzmittel; S. Reichenberger, FOOTWAYS SAS; K. Thomas, Institute for Agroecology, M. Tripp, RLP AgroScience, IA / Institute for Agroecology For thegeo based model FOCUS Surface Water was established based on the principle “one safe use” in Europe. Although FOCUS Surface Water has been used for regulatory decision making the overall level of protection is not clear. That includes in particular the level of representativeness of the FOCUS Surface Water scenarios for the national specific agro-environmental conditions. To answer this question for the national authorisation in Germany as well as to adjust the German national exposure and risk assessment procedure to the FOCUS Surface Water approach in the harmonisation spirit of regulation (EC) 1107/2009 and furthermore to enhance some of the limitations of FOCUS Surface Water the German Federal Environment Agency (UBA) has launched in 2011 a research project to develop a national Geo-based [German] Runoff, Erosion, and Drainage risk Assessment. GERDA will replace FOCUS Surface Water and EXPOSIT to evaluate the surface water exposure from PPP input by runoff, erosion and drainage in future. The FOCUS based but nationally specified exposure assessment approach might be applicable to EU member states. Long-term PEC time series (30 years) for surface water and sediment are calculated, in contrast to only 12 or 16 months in FOCUSSw. Edge-of-field loss is still calculated by PRZM and MACRO but instead of TOXSWA, GERDA uses the model STEPS 1-2-3-4 to calculate PEC hourly time series in the receiving water body. To select a reliable soil-climate scenarios PRZM and MACRO simulation runs for soil/climate/crop/substrate-combinations, covering the entire agricultural land area (arable and permanent crops) are used to calculate long-term PECsw time series. The time series are ranked according to the calculated PECsw to create a area-weighted CDFs. The level of protection of the exposure endpoint (PEC) will be known. For Germany GERDA calculations are based on a combination of 80th spatial / 80th temporal percentile for national specific soil-climate-scenario leading to an “overall” (approx.) 90th centile level of protection. Details on the concept and the main components have already been presented. GERDA runs on 13 test cases for each of the three different water bodies ditches, streams and ponds. The results of the single country and the whole Northern Zone scenarios will be presented. Therefore insight in the representativeness and the protectiveness of these scenarios is key to building confidence in the risk assessments being made, both at the national and zonal levels. Furthermore, insight into the representativeness and the protectiveness of these scenarios may assist in harmonising the zonal procedure of the risk assessment for pesticide leaching to groundwater. To assess the representativeness of the Northern groundwater scenarios an analysis of the soil and climatic characteristics of these scenarios was conducted. These characteristics were compared with the characteristics of the detailed Footways agro-pedo-climatic combinations covering the Northern zone countries. The analysis focused on climate and soil aspects and was conducted both at the national and zonal levels. Overall, the representativeness of the FOCUS/national scenarios for the agricultural area of the Northern Zone countries is low. However, what is important for the risk assessment is protectiveness (i.e. the cumulative worst-case-ness) and not representativeness. To assess the protectiveness of the Northern groundwater scenarios twelve example applications, comprising 12 active substances (both hypothetical and real ones) and 6 metabolites, were defined. The applications were simulated according to the national requirements in the countries in the zone with the PEARL, PELMO and MACRO model. Results obtained for the FOCUS/national scenario modelling were compiled in a table containing overall and annual pesticide leaching concentrations and leaching fluxes for 450 unique combinations of simulated compound, scenario, model and output depth (162 combinations for MACRO, 144 for PEARL and 144 for PELMO). These results were plotted over the spatial cumulative distribution functions (CDFs) of the various leaching endpoints to determine the cumulative worst-case-ness, i.e. protectiveness, of the FOCUS/national scenarios for the individual countries and the whole Northern Zone. The positions of the FOCUS/national scenarios on the national and zonal CDFs of the various leaching endpoints, notably the 80th percentile annual PECgw, for the 18 simulated compounds will be presented.

53 Surface water risk assessment of pesticides in Ethiopia B. Teklu, Wageningen University / Environmental Sciences Aquatic Ecology and Water Quality Management Group; P.I. Adriaanse, Alterra Wageningen University and Research Centre / ERA team; M. ter Horst, Alterra, WUR; J.W. Deneer, Alterra Wageningen University and Research Centre / ERA team; P.J. van den Brink, Alterra and Wageningen University

Scenarios for future use in the pesticide registration procedure in Ethiopia have been devised for 3 separate Ethiopian locations, which are considered to be

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use those CF, EF should be geographically localised (inventory spatialisation, IS). The literature review allows for the identification of 111 recommendations and 36 approaches on the spatial dimension integration in LCA (44 references). They are classified based on their objectives (InR, IS, ImR, integration during impact calculation and interpretation phase) and application in LCA databases and software. The literature review reveals that LCA practitioners have used most of the approaches for ImR. Is it a new practice even though the geographic information needed is often easily accessible. The additional workload to integrate the spatial dimension depends on the uncertainty level that the practitioner wants to reach. However, some sectors (e.g. the agricultural sector) and impact categories (e.g. land use) require a specific attention for ImR and IS. Ideally, geographical data should be used, but it requires some skills in Geographic Information System tools. An alternative approach is to use aggregated data or CF enriched with uncertainty related to spatial variability. Practical guidelines are provided in order to help LCA practitioners to deal with spatial variability. This research points out the need for a consistent operational methodology allowing to prioritise the effort of regionalisation in LCA depending on the decision context.

56 Input-output based life cycle assessment of production and consumption in Belgium - Comparison of three Belgian regions based on regionalised input-output data
V. Zeller, Université Libre de Bruxelles; M. Degrez, ULB / MAT

Most life cycle assessments (LCA) or footprint studies that focus on production and use of crop inputs in order to provide a more accurate estimate of environmental impacts related to the primary sector, the Walloon region in the manufacturing sector, and Brussels in the service sector and household consumption. The results show that for this case study, the regionalisation of input-output data improves accuracy of results. In order to draw safe conclusions the analysis must be conducted for other years and include a detailed product resolution (ongoing research).

57 Conceptual approach and operational tool for consistent calculation of temporally differentiated LCIs
L. Tiruta-Barna, Université de Toulouse / INSA UPS INP LISBP; Y. Pinge, Normandy University / LITIS; T. Navarrete-Guitierrez, Public Research Centre Henri Tudor; E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE

Conventional Life Cycle Inventories (LCIs) are static models of product systems, assuming steady state operation of unit processes and neglecting time lags and stocks of products between supply and demand. As a result, the aggregation of environmental interventions without proper consideration of the time dimension cannot be based on static LCIs. Assumptions results -We aim to present a new conceptual and computational framework for the consideration of time dependency in LCIs. Process modelling is used to describe the production flows and environmental interventions of each unit process and supply modelling is used to include time lags and stocks on the raw materials exchanged by unit processes of the product system. The combination of production and supply models in life cycle networks is based on the approach (time dimension dependency). The new approach allows for the consideration of time dependency in each node. For the computation of time dependent LCI, i.e. of the time resolved environmental interventions, a tracking (graph search) algorithm is proposed. To this aim, a Web software was successfully developed. A test bed case illustrating the effectiveness of the approach was run in a computational environment and solved both analytically and numerically. The computational requirements and additional data and information needs for the application of the tool to full scale life-cycle networks are discussed.
58 Impact assessment meets global sensitivity analysis techniques: misunderstandings, advantages and unexplored potential
S. Cucurachi, Institute of Environmental Sciences(CMLS) / CML Industrial Ecology; E. Borognomia, Bocconi University / Management Science Laboratory; R. Heymans, Leiden University / Faculty of Economics and Business Administration Department of Econometrics.

The trustworthiness of LCA as a decision support tool has certainly improved, as its robustness as a field of science. Improvements have especially regarded the impact assessment methods that are used in the LCIA phase. LCIA methods of ever increasing complexity have been developed to deal with a great number of material and immaterial stressors. LCIA methods are now able to capture local differences and provide a spatially-expansive characterisation of emissions, and are also able to consider temporal dynamics. Such improvements have contributed to strengthen LCA. However, the elephant in the room still remains the matter of uncertainty.

Even though the topic has attracted the attention of developers since the early days of LCA and still comes high in the agenda of meetings and conferences, the methodological proposals to deal with uncertainty analysis have often been neglected in the practice. Together with uncertainty analysis, other statistical techniques have failed to attract the attention of LCA experts. Techniques of global sensitivity analysis are a remarkable example of this trend. Although the literature on the subject is vast and rich of causes for reflection, the literature shows that until now that the LCA world has almost singlehandedly ignored the newest developments in the field. The sheer definition of sensitivity analysis appears to be obsolete in the LCA tradition: it is indeed an interpretation of values mixed up with uncertainty analysis, or used as a synonym of scenario evaluation. Trying to clarify all misinterpretations and inconsistencies, in this contribution we define a stepwise protocol that uses uncertainty analysis in combination with an ensemble of global sensitivity analysis techniques to fully analyse LCIA models.

59 The social footprint - A practical approach to comprehensive and consistent social LCA
B.P. Weidema, Aalborg University / Danish Centre for Environmental Assessment

The term “social” is here understood as it is used in welfare economics, to signify the impact of an activity on living conditions of humans, to the extent that this affects the benefits and costs of an activity. It indicates thus the relationship between bioeconomic impacts and society. A streamlined approach to importance of impact categories - explicit characterisation of emissions, and are also able to consider temporal dynamics. Such improvements have contributed to strengthen LCA. However, the elephant in the room still remains the matter of uncertainty.

Even though the topic has attracted the attention of developers since the early days of LCA and still comes high in the agenda of meetings and conferences, the methodological proposals to deal with uncertainty analysis have often been neglected in the practice. Together with uncertainty analysis, other statistical techniques have failed to attract the attention of LCA experts. Techniques of global sensitivity analysis are a remarkable example of this trend. Although the literature on the subject is vast and rich of causes for reflection, the literature shows that until now that the LCA world has almost singlehandedly ignored the newest developments in the field. The sheer definition of sensitivity analysis appears to be obsolete in the LCA tradition: it is indeed an interpretation of values mixed up with uncertainty analysis, or used as a synonym of scenario evaluation. Trying to clarify all misinterpretations and inconsistencies, in this contribution we define a stepwise protocol that uses uncertainty analysis in combination with an ensemble of global sensitivity analysis techniques to fully analyse LCIA models.

60 The water footprint of consumption - tracking origins and impacts through multi-regional input output analysis (MRIO)
S. Pfister, ETH Zurich; S. Lutter, Vienna University of Economics and Business; M. Mekonnen, Millenium-Energy.

Global agriculture is the main driver of water consumption and related environmental impacts and its products are part of a highly globalized market. Therefore, we used the enhanced EXIOPOL-multiprovincial input output (MRIO) dataset created in the CREEA project. It is accounting for water consumption and trade of 43 countries (representing ~95% of the global GDP) and 5 rest-of-the-world (RoW) regions with a total of 169 industrial sectors and 200 product groups in each country/region facilitating a proper water footprint assessment of final consumption. Since water consumption calculations have high uncertainties we developed the CREEA data for evaluating whether the activities remained within the stipulated “contamination layer cap. This reduces available but not total concentrations. This knowledge proved important spreading and transport routes for the PAHs in the EU27 for its final consumption is reported outside full a LCA and the outcomes are compared. The “blue water” footprint of EU27 final consumption of products is mainly located in Europe, the US, China, India, Pakistan, Brazil, Egypt and Thailand. The scarce water originates mainly from Europe, India, Pakistan, the US, China, and Egypt. For green water Sub-saharan Africa and Latin America have a much higher contribution, indicating high land use impacts caused by EU27 production. Interestingly, the share of green, blue and grey water consumption is roughly between one to one third of the total. This shows the high dependency of EU states on foreign land and water resources. The main sectors contributing to the water footprint is agricultural production, mainly imported as processed bio-based products through other sectors, incl. processed food and leather. The high uncertainty of water consumption values for crop production is reflected by high confidence intervals even for EU27’s allocative methods (1,9). Large discrepancies in EU27 water footprint origins are observed for the African and Asia/Pacific regions, but the overall picture is not differing a lot.

Bioavailability of organic chemicals: Linking science to risk assessment and regulation

61 Towards a unified approach for the determination of the bioaccessibility of organic pollutants
C.D. Collins, Reading University / Soil Research Centre; S. Alcega-Garcia, University of Reading; M. Crags, University of Reading / Department of Geography and Environmental Science; K. Kademoglou, University of Reading / geography and Environmental Sciences; S. Lowe, University of Reading.

Bioaccessibility studies have been widely used as a research tool to determine the potential human exposure to ingested contaminants. More recently they have been practically applied for soil borne toxic elements. This paper reviews the application of bioaccessibility tests for characterising the environmental fate of organic pollutants and contaminated matrices. Important factors are reported to be: the physiological relevance of the test, the components in the gut media, the size fraction chosen for the test and whether it contains a sorptive sink. The bioaccessibility is also a function of the composition of the matrix (e.g. organic carbon content of soils) and the physicochemical characteristics of the chemical under test. Despite the widespread use of these methods they are a large range of test that have few validation studies with animal models. We propose a unified protocol for a bioaccessibility test for organic pollutants. The robustness of the test should first be confirmed through inter laboratory comparison, then tested in-vivo.

62 Use of bioavailability concepts and passive samplers in “real-world” projects in Norway

In Norway regulation opened up for the inclusion of bioavailability concepts relatively early (around 2007). This has allowed us to include bioavailability in a number of projects on the border of science, consultancy and regulation. Three projects right from the limelight of public interest will be presented: at the hydropower plant of Hakavika, Norway, a series of sand blasting activities have resulted in the contamination of the surrounding soil with PAHs from the pipe coatings. A thorough risk assessment was undertaken in order to assess the most important spreading and transport routes for the PAHs in order to suggest the most appropriate remediation method. The site specific soil-water partitioning coefficients were determined to be 250 to 4000 times higher than validated used in the model as default parameters. This means that PAHs in this particular soil were much more strongly bound to the soil than the risk assessment model assumed. The PAHs are therefore much less available to organisms and to leach to the surrounding soil, than estimated from the standard values. This knowledge proved especially useful in the selection of the most suitable remediation method. In the Osfjord, Norway, a large-scale clean-up of the whole harbour was carried out during 2008-2012. The clean-up consisted of large-scale removal of contaminated sediments, followed by disposal in an adjacent deep-water disposal site. Continuous monitoring of freely dissolved, bioavailable concentrations aided the authorities in evaluating whether the activities remained within the stipulated “contamination budgets”. In the Grenlandfjords, Southern Norway, the authorities are trying to find solutions for the water pollution in the fjords. Innovative methods were deployed to estimate risks, such as benthic flux chambers, passive samplers and compartment fate models. A large-scale remediation project was set up where activated carbon was added as a thin-layer cap. This reduces available but not total concentrations, so that availability concepts were needed to convince the authorities that this was a valid remediation option. Thin layer capping with activated carbon showed an enormous effectiveness in time, up to 85% reduction in fluxes after 5 years, up to 90% reductions in bioavailability (both chemical via passive samplers and biological via bioaccumulation. Including bioavailability concepts has helped in important and large-scale risk assessment and remediation projects.
The "biological" component of bioavailability: the usage of ecotoxicological tests

J. Roemibe, ECT Oekotoxikologie GmbH

In 2008, the International Organisation for Standardisation (ISO) published a guide for ecotoxicological test methods useful for the determination and the assessment of bioavailability in the soil compartment. As examples I will focus on soil invertebrates and, to a lesser degree, on plants, mainly because they are relatively well studied and they can be used both as effect as well as accumulation indicators. Because of the high number of organic chemicals (which may end up in soils), the large differences in soil properties (which may influence the availability of these chemicals) and the taxonomic, physiological and behaviour diversity of soil organisms (which may react quite differently), it is obvious that there is not one / best test method to be recommended: in any case a test battery is needed, consisting of methods which reflect the various combinations between chemicals, soils and organisms, especially the different exposure pathways. Of course, this does not mean that these tests have always to be used -- but different standardized methods must be available when assessing the bioavailability of a given organic chemical in soil. In addition, I will briefly discuss whether there are still gaps in test methodology. But more importantly, criteria are needed in order to identify those tests which are most suitable and practical for addressing the bioavailability of chemicals in a regulatory context, preferably as part of existing test strategies. One example of an existing test strategy which has extensively implemented bioavailability, is the TRIAD approach (currently being standardized by ISO). Possible testing requirements of this approach will be presented and discussed.

Bioavailability and REACH

B.J. Versonen, European Chemicals Agency - ECHA / Evaluation; C. Aja, ECHA-European Chemicals Agency

REACH, the European Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals requires so-called 'standard information requirements' to be provided by manufacturers and importers of chemicals in the EU. These include a test battery for determining bioavailability, the 'chemical safety assessment'. Under the REACH regulation, worst-case assumptions are often used as in a first tier risk assessment. This is also true for bioavailability where 100% bioavailability is initially assumed. However, bioavailability concepts are often used by registrants to refine risk assessment, e.g. in calculating a first tier assessment, or for certain endpoints as (part of) justifications to waive testing that is part of the information requirements under REACH. Although the best known bioavailability concepts have been developed for data rich metals, similar approaches are used for organic chemicals and non-metal inorganic substances. REACH explicitly allows for such substance-specific approaches for bioavailability to be used by registrants to deviate from the standard testing regime if a number of conditions are fulfilled. Apart from the scientific validity of these approaches, they for example need to be well documented and justified in the dossier and the increased or decreased uncertainty related from the approaches needs to be documented. In this presentation examples of approaches that have been used under REACH will be used to 1) illustrate aspects which are of specific importance in a regulatory context, 2) explain possible pitfalls of bioavailability approaches, 3) identify issues which need further scientific developments.

Classification and modelling of non-extractable residue (NER) formation from pesticides in soil

M. Kistner, Helmholtz centre for environmental research - UFZ / Department of Environmental Biotechnology; K.M. Nowak, RWTH Aachen University / Institute for Environmental Research Biology; V. Almster; R. Trapp, Danmark Tekniske Universitet / DTU Environment; A. Schaeffer, RWTH Aachen University / Institute for Environmental Research

The presentation provides a comprehensive overview about the formation of non-extractable residues (NER) from organic pesticides and contaminants in soil and tries classifying the different types. Anthropogenic organic chemicals are deliberately (e.g. pesticides) or unintentionally (e.g. polyaromatic hydrocarbons [PAH], chlorinated solvents, pharmaceuticals) released in major amounts to nearly all compartments of the environment. Soils and sediments as complex matrices provide a wide variety of binding sites and are the major sinks for these compounds. Many of the xenobiotics enter soil undergo turnover processes and can be volatilised, leached to the groundwater, degraded by microorganisms or taken up and enriched by living organisms. Xenobiotic NER may be derived from parent compounds and primary metabolites that are sequestered (sorbed or entrapped) within the soil organic matter (type I) or can be covalently bound (type II). Both types may pose a considerably environmental risk of potential release. However, NER resulting from elevated biodegradation, which means the conversion of carbon (or nitrogen) from the compounds into microbial biomass molecules during microbial degradation (type III, bioNER), do not pose any risk. Experimental and analytical approaches to clearly distinguish between the types are provided and a model to prospectively estimate their fate in soil is proposed.

Bioavailability of organic chemicals: How to link science to risk assessment and regulation

J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiología / Agroquímica y Conservacion del Suelo / LI. Risk assessment, Alterra, Wageningen UR / CALM; I. Parsons, University of Amsterdam / IBED

Bioavailability of organic chemicals: How to link science to risk assessment and regulation. Jose-Julio Ortega-Calvo (IRNAS-CSIC. ES). Joop Harmens (Alterra/Wageningen UR, NL) and John R. Parsons (University of Amsterdam, NL) After more than 20 years of research, the science on bioavailability of organic chemicals is mature enough to be used by regulators and industry. However, this application is still missing. To facilitate the implementation, a SETAC Europe Special Science Symposium has been organized in October 2014. Key speakers coming from industry, regulation and academia presented their few, which facilitated the discussion in break out groups. The goal was to achieve a clear consensus approach among scientist, regulators and industry on how to think on and how to apply bioavailability. For regulation of chemicals (REACH) a prospective few is necessary, as prescribed in European regulation. Bioavailability can have a role in this, but discussions are more leaded by the non-bioavailable part and especially the part that is non extractable (NER). Should this part be considered as non-toxic or as a chemical time bomb. On national level the retrospective few on bioavailability is more important and can have a role in management of contaminated sites. Science offers chemical and biological methods, that are even partly standardized. Methods are available to measure the available part in the pore water and also the potential available part. Using bioassays the effect of the bioavailable fraction becomes measurable. No methods are available to measure the Non Extractable Residue or bound residues. Important result of the discussion was that the communication should be an important role in the implementation of science is able to explain bioavailability, but does not realize that regulations needs measurement data to enable decision making. It was concluded that communication between science and regulation should focus on the parts that are bioavailable (=measurable) and also on the part that is not bioavailable, which is the total measurable amount minus the available amount. It has to be made clear that with these measurable fraction, the non measurable part can be explained and that the non-measurable fraction do not cause any risk. Defining only the bioavailable and the non-bioavailable part for regulation, the still going on scientific discussion on definitions can be limited to the scientific world.

Ecological Consequences of Exposure to Pharmaceuticals (II)

Accumulation, Distribution, Metabolism and Elimination (ADME) of fluoxetine in a model passerine bird


The effects and pharmacology of pharmaceuticals are extensively studied in model organisms. The availability of these chemicals is mature enough to be used in the scenarios of birds ingesting invertebrates from the trickling filters of wastewater treatment plants which are contaminated with the commonly prescribed antidepressant fluoxetine. The hypothesis for mammalian to avian systems was assessed. Previously we have found an environmentally relevant dose of fluoxetine for starlings has the potential to affect fitness related traits. Following 22 weeks of dosing wild-caught starlings in captivity with fluoxetine contained in spiked invertebrates to mimic environmental uptake, dosing was stopped and birds were euthanized 2, 26, 50 and 74 h after the final dose. Internal concentrations (brain, kidney, liver and muscle) and excretion levels of fluoxetine and metabolites were measured using methods including LC-MS/MS and FT-ICR-MS. The internal concentrations at the different time points were used to determine accumulation, distribution, metabolism and elimination (ADME) for starlings. Accumulation was found to be low with tissue concentrations in the range of 3.1±4.4 and 0.2±2.5 ng/g for fluoxetine and norfluoxetine respectively with concentrations highest in the liver and relatively low in the brain. Elimination half-lives were rapid, typically 3-10 hours. Compared with ADME data for humans and model mammals, our results suggest that avian to rodent but not avian to human read-across provides the best support for the use of read-across in avian pharmaceutical risk assessments. The relatively poor match between human and avian ADME data is suggested to be due to differences
between in both metabolic pathways and basal metabolic rate. It is suggested that the low level of accumulation and rapid elimination of fluoxetine and its active metabolite in starlings means that, in order for the effects observed under experimental conditions to occur in free-living birds, uptake of fluoxetine would have to be high and constant and receptors would have to be more sensitive than in humans.

68 Exposure of griffon vultures to fluoroquinolones in NE Spain


Veterinary drugs that are commonly used in livestock production can remain as residues within the carcasses of animals after death. As a result, various scavengers, including vultures, may be exposed to a range of potentially bio-active compounds – the implications of which remains very poorly characterised. Across Asia, exposure to diclofenac (a non-steroidal anti-inflammatory drug) via this pathway has caused the near global extinction of at least three species of Old World Gyps vultures. This highlights the urgent need for far better ecopharmacovigilance and the requirement for more comprehensive life-cycle assessments for pharmaceutical products currently in use. Despite the observed impact of NSAIDs in Asia on scavengers, surprisingly little monitoring data currently exists regarding the fate of pharmaceuticals in the environment and their potential effects on higher wildlife. European vultures provide an obvious starting point for further work; hence, we present data regarding the exposure of griffon vultures (Gyps fulvus) to antibiotics in Aragon, NE Spain. Griffon vultures (n=492) were captured at 19 feeding sites in Aragon (NE Spain) between 2008 and 2011. Blood samples were analysed by LC-ESI/APICI-MS. Fluoroquinolones were detected in 15.2% of the plasma samples. Positive samples were detected in half of the “vulture restaurants”, which indicates that not all feeding sites were taking in carcasses containing fluoroquinolone residues. However, at positive sites the percentage of samples with enrofloxacin/ciprofloxacin was as high as 73.5%. With the exception of one sampling site, enrofloxacin (13%) was more frequently detected that ciprofloxacin (7.4%), which may reflect recent exposure in livestock and vultures as ciprofloxacin is a metabolite of enrofloxacin. The mean (±SD) level of enrofloxacin was 6.5±1.0 ng/ml, for ciprofloxacin was 6.4±1.2 ng/ml and for total fluoroquinolones was 12.9±1.7 ng/ml. However, log-transformed concentrations revealed a higher value for enrofloxacin than ciprofloxacin (geometric means = 2.0 vs. 0.2 ng/ml). Further research will be needed to evaluate whether there are potential adverse effects on vultures of exposure to antibiotics through medicated livestock, but the data indicate that vultures are regularly being exposed to pharmaceutical residues and this may be of concern in relation to the potential exposure to more toxic compounds such as NSAIDs.

69 New Approach to Assess the Effects of Veterinary Medical Products (VMPs) on Aquatic and Soil Organisms under Field Conditions: Experiences with Sheep Dung

B. Förster; A. Coors, ECT Oekotoxikologie GmbH; R. Düring, Justus Liebig University Giessen / Institute of Soil Science and Soil Conservation Research Centre for Biosystems Land Use and Nutrition IPZ; M. Ebke, MESOCOSM GmbH Institut für Gewässerschutz; N. Graf, A. Kulenko, University of Giessen; A. Schlegel, M. Wohde, Institute of Soil Science; J. Römhke, ECT Oekotoxikologie GmbH

Veterinary medical products (VMPs) are widely used in today's livestock farming. According to the guidelines published by the Veterinary International Conference on Harmonization (VICH) (2000, 2004), ecotoxological data are required within the registration process of VMPs. For highly effective parasiticides, the environmental risk assessment includes a comprehensive evaluation of their effects on both higher aquatic and soil organisms. If concerns are identified in laboratory tests with VMPs currently in use. Despite the observed impact of NSAIDs in Asia on scavengers, surprisingly little monitoring data currently exists regarding the fate of pharmaceuticals in the environment and their potential effects on higher wildlife. European vultures provide an obvious starting point for further work; hence, we present data regarding the exposure of griffon vultures (Gyps fulvus) to antibiotics in Aragon, NE Spain. Griffon vultures (n=492) were captured at 19 feeding sites in Aragon (NE Spain) between 2008 and 2011. Blood samples were analysed by LC-ESI/APICI-MS. Fluoroquinolones were detected in 15.2% of the plasma samples. Positive samples were detected in half of the “vulture restaurants”, which indicates that not all feeding sites were taking in carcasses containing fluoroquinolone residues. However, at positive sites the percentage of samples with enrofloxacin/ciprofloxacin was as high as 73.5%. With the exception of one sampling site, enrofloxacin (13%) was more frequently detected that ciprofloxacin (7.4%), which may reflect recent exposure in livestock and vultures as ciprofloxacin is a metabolite of enrofloxacin. The mean (±SD) level of enrofloxacin was 6.5±1.0 ng/ml, for ciprofloxacin was 6.4±1.2 ng/ml and for total fluoroquinolones was 12.9±1.7 ng/ml. However, log-transformed concentrations revealed a higher value for enrofloxacin than ciprofloxacin (geometric means = 2.0 vs. 0.2 ng/ml). Further research will be needed to evaluate whether there are potential adverse effects on vultures of exposure to antibiotics through medicated livestock, but the data indicate that vultures are regularly being exposed to pharmaceutical residues and this may be of concern in relation to the potential exposure to more toxic compounds such as NSAIDs.

70 A comparison of aquatic species responses to anticancer drug exposure

G. Harris, C.A. Harris, J.P. Sumpter, Brunel University / Institute for the Environment, London.

Classic anticancer drugs differ from the majority of other classes of pharmaceuticals in that they are specifically designed to damage cells. Several of these drugs have been measured in the environment, albeit at extremely low concentrations. However, relatively little research has been conducted on their impact on aquatic organisms. The purpose of this study was to assess whether aquatic species are adversely affected by cytotoxic drugs, and if so, at what concentrations. Six compounds were selected for the study: 5-Fluorouracil (5FU), Cyclophosphamide (CYP), Cisplatin (CIS), Carboplatin (CAR), Hydroxyurea (HYD) and Imatinib (IMB). A microalgae (R. subcapitata), an invertebrate (D. magna) and a vertebrate (D. rerio) were chosen as representative species from different phylogalms and were exposed to the anticancer drugs following the relevant OECD guidelines. Algae and daphnia were exposed to the six drugs, both singly and as two simple mixtures. Zebrafish were exposed to the first three of these drugs, also singly and as a simple mixture. Growth inhibition of the microalgae was observed for all drugs singly (apart from CAR) with EC50 values ranging from 0.44 mg/l for 5FU to 3790 mg/l for CYP. It was not possible to generate EC50 values for CAR under these conditions. Nevertheless, algae at a concentration of 10 mg/l for CAR showed no growth inhibition nor did daphnia at a concentration of 50 mg/l for CAR. However, daphnia showed a high sensitivity to the drugs tested. This sensitivity increased with increasing concentrations up to an EC50 of 1 mg/l for CYP. For CAR, invertebrates were less sensitive. No effects were observed towards the paraciticide. Dung beetles were less sensitive. No effects were observed on soil arthropods. Decomposition of dung, measured as ash-free mass loss over time, was very fast. It seems to be partly affected by the paraciticide. The methodological details and the results of this field study will be discussed in the light of the applicability of this approach for a higher tier assessment of effects of VMP in sheep dung on soil invertebrates.

71 Effects of antibiotics on the growth and physiology of green algae, cyanobacteria and diatoms


A wide range of antibiotics have been detected in the aquatic environment worldwide. Algae, as non-target organisms and primary producers in the environment, are very sensitive and vulnerable to antibiotics. Once algae are exposed to antibiotics, ecosystem processes may be potentially disrupted due to their primary producer status in the food chain. The aims and objectives of this study were: 1) to compare the responses of diatoms, green algae and cyanobacteria to commonly detected antibiotics (tylosin, lincomycin and trimethoprim) using standard test methods for effects on growth; 2) to explore the effects of antibiotic mixtures on selected algae species and evaluate the predictive capabilities of CA and IA models for estimating mixture effects; 3) to explore the effects of the three antibiotics on the physiology of the four most sensitive species identified in 1), including changes in photosynthesis, in nitrogen assimilation and in photosynthetic pigment synthesis and how antibiotics affect the acclimation of the algae to light. Currently most approaches for assessing risks of pharmaceuticals only use a single green algae species and/or cyanobacteria species. This study demonstrates large differences in sensitivity both across algae groups and within algae groups and these differences seem to be substance specific. This study also proves that the photosynthetic process can be damaged by antibiotics by reducing light utilisation efficiency (one potential reason). As the photosynthetic process is the primary mode of energy metabolism in algae, we would advocate that detailed physiological studies should be undertaken to reveal the damage mechanism. Key words: algae, antibiotics, ecotoxicology, physiology

72 Behavior modifications: bridging the gap between laboratory assays and natural behavior in Ecotoxicological risk-assessment studies


Horseradish peroxidase (HRP) has shown great promise as a reporter of stress responses in several invertebrates. However, relatively little research has been conducted on their impact on aquatic organisms. The purpose of this study was to assess whether aquatic species are adversely affected by cytotoxic drugs, and if so, at what concentrations. Six compounds were selected for the study: 5-Fluorouracil (5FU), Cyclophosphamide (CYP), Cisplatin (CIS), Carboplatin (CAR), Hydroxyurea (HYD) and Imatinib (IMB). A microalgae (R. subcapitata), an invertebrate (D. magna) and a vertebrate (D. rerio) were chosen as representative species from different phylogalms and were exposed to the anticancer drugs following the relevant OECD guidelines. Algae and daphnia were exposed to the six drugs, both singly and as two simple mixtures. Zebrafish were exposed to the first three of these drugs, also singly and as a simple mixture. Growth inhibition of the microalgae was observed for all drugs singly (apart from CAR) with EC50 values ranging from 0.44 mg/l for 5FU to 3790 mg/l for CYP. It was not possible to generate EC50 values for CAR under these conditions. Nevertheless, algae at a concentration of 10 mg/l for CAR showed no growth inhibition nor did daphnia at a concentration of 50 mg/l for CAR. However, daphnia showed a high sensitivity to the drugs tested. This sensitivity increased with increasing concentrations up to an EC50 of 1 mg/l for CYP. For CAR, invertebrates were less sensitive. No effects were observed towards the paraciticide. Dung beetles were less sensitive. No effects were observed on soil arthropods. Decomposition of dung, measured as ash-free mass loss over time, was very fast. It seems to be partly affected by the paraciticide. The methodological details and the results of this field study will be discussed in the light of the applicability of this approach for a higher tier assessment of effects of VMP in sheep dung on soil invertebrates.
natural context are crucial for proper risk assessment of aquatic contaminants. In this study, we have for the first time being able to closely match behavioral modifications induced by an aquatic contaminant (Oxazepam) measured in laboratory assays to the in situ behavior of a wild Eurasian perch (Perca fluviatilis) in their natural environment. By using micro-scale acoustic telemetry we were able to track the behavior of fish over an entire lake with very high temporal and spatial resolution. Eurasian perch exposed to the anxiolytic drug Oxazepam were compared to unexposed fish in ecologically important parameters such as interaction with predators, social behavior, activity patterns, diurnal cycles, personality dynamics, as well as survival. Results are discussed from an ecological and conservational perspective. The presentation will include powerful visuals illustrating the way acoustic telemetry can be used to study behaviorally mediated effects of pharmaceuticals and other contaminants in the aquatic environment.

### Fish model species in environmental toxicology (II)

73 A new approach to assess sediments toxicity with Danio rerio embryos

R. Massig, Effect Directed Analysis; D. Kurth, Helmholtz centre for environmental research - UFZ / Department of Effect Directed Analysis; S. Scholz, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; S. Katsiadaki, Carlos III University of Madrid; S. Cefas / Environment and Animal Health; M. Krauss, T. Schulze, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; M. Santos, University of Exeter; T. Uren Webster, University of Exeter / Biosciences College of Life and Environmental Sciences

**Effects of hypoxia on the toxicity of copper in two model fish species, Danio rerio and Gasterosteus aculeatus L.**

J. Fitzgerald, University of Exeter; R. Wilson, Exeter University; L. Katsiadaki, Cefas / Environment and Animal Health; E.M. Santos, University of Exeter / Biosciences College of Life and Environmental Sciences

Hypoxia is a global and increasingly important stressor in aquatic ecosystems, with major impacts on biodiversity worldwide. Hypoxic waters are often contaminated with chemicals but, despite this, little is known about the interactions between hypoxia and chemical stressors. Previous studies have demonstrated some remarkable alterations in the toxicity of some compounds when exposures occur under hypoxia. However, current knowledge spans only a few chemicals and species, and has incompletely investigated the life cycle of aquatic organisms, highlighting a major knowledge gap for evaluating the environmental impact of chemicals, where exposure often occurs under reduced oxygen. It is critical to investigate the interactions between these stressors in order to more effectively and objectively determine realistic safety thresholds for contaminants in aquatic ecosystems. Here, we started this issue by investigating if hypoxia affects the toxicological response to copper in zebrafish (Danio rerio) and three-spined stickleback (Gasterosteus aculeatus L.).

**74 Effects of hypoxia on the toxicity of copper in two model fish species, Danio rerio and Gasterosteus aculeatus L.**

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**Fish model species in environmental toxicology (II)***

75 The 3-spined stickleback as a model for endocrine disruption and mixtures toxicity

L. Katsiadaki, Cefas / Environment and Animal Health; P. Antczak, University of Liverpool / Institute of Integrative Biology; T. Williams, University of Birmingham / School of Biosciences; M. Sebire, Cefas; M. Sanders, Cefas / Environment and Animal Health; J. Elphistone Davis, Cefas Weymouth / Environment and Animal Health; R. Van Aarle, University of Exeter / Biosciences College of Life and Environmental Sciences; J. Chapman, The University of Birmingham / School of Biosciences; F. Falciani, University of Liverpool; A.P. Scott, Cefas Weymouth / Environment and Animal Health

The three-spined stickleback has a number of advantages as a model species which are particularly useful in the fields of endocrine disrupting chemical (EDC) research and regulation. These advantages include unique natural traits which are under hormonal control, such as the androgen biomarker protein spiggin (Katsiadaki et al., 2002), an elaborate reproductive behaviour, strong sexual selection and a sex determination system that is not influenced by environmental factors, other than EDCs. Over the past 15 years an impressive EDC-specific toolbox has been developed for this species. Techniques developed include in vitro fertilisation, DNA sex marker, ELISA’s for vitellogenin and spiggin, methods for global gene and metabolite expression whilst international test guidelines and guidelines with short life cycles offer significant advantages allowing the assessment of the rapid impacts of chemical stressors on parameters directly relevant to population level effects. In this context, we have employed both a laboratory model fish species (zebrafish; Danio rerio) as well as a native species to UK freshwaters (brown trout; Salmo trutta) to understand the mechanisms of chemical toxicity using a combination of genomics, physiological, biochemical and histological analysis, and a range of in vivo studies. The approach of using both the common species, zebrafish as a surrogate model, Danio rerio as a native species to UK freshwaters (brown trout; Salmo trutta) to understand the mechanisms of chemical toxicity using a combination of genomics, physiological, biochemical and histological analysis, and a range of in vivo studies. The approach of using both the common species, zebrafish as a surrogate model, Danio rerio as a native species to UK freshwaters (brown trout; Salmo trutta) to understand the mechanisms of chemical toxicity using a combination of genomics, physiological, biochemical and histological analysis, and a range of in vivo studies. The approach of using both the common species, zebrafish as a surrogate model, Danio rerio as a native species to UK freshwaters (brown trout; Salmo trutta) to understand the mechanisms of chemical toxicity using a combination of genomics, physiological, biochemical and histological analysis, and a range of in vivo studies.
Systems Biology: increasing the capacity, understanding ecosystems and response mechanisms to model chemicals (including nanomaterials) (Systems biology)

78 The use of High-Throughput screening technologies in Daphnia magna to identify environmental hazards of contaminants
B. Campos, IDAEA-CSIC / Environmental Toxicology; J. Colbourne, University of Guelph; A. Sittinger, Indiana University / Biology; D. Fletcher, Agilent Technologies; R. Tauler, IDAEA-CSIC; B. Pina, IDAEA-CSIC / Environmental Chemistry; C. Barata, CSIC / Environmental Chemistry

Recently, we are observing several changes in regulatory paradigms. Nowadays, the main interest of risk assessment is the full characterization of risk across different biological, organismal levels on a systems biology approach. The development of Adverse Outcome Pathway concept in ecotoxicological research provides a stepping stone for the use of alternative testing methods for hazard assessment, allowing to related molecular signatures of initiating events with apical effects of regulatory relevance. With the recent development of high-throughput sequencing technologies and "Omics" derived assays these objectives are now within our reach. We present the development and validation of a new, more complete and public microarray of Daphnia magna and its comparison with results from RNaseq. We will present a pipeline for data analyses and interpretation, which will be useful in the description of molecular initiating events, and a chemometric tool data analysis to identify de-regulated genes and link them with apical and regulatory relevant effects. For the development of the gene models, 7 billion mRNA reads (the most information intensive effort until this moment) were used in the frame of the EVIGENE project. These gene models were then used as template in eARRAY platform. Validation was performed using 6 developmental stages of females, 1 adult males and 3 contaminated exposed animals. RNAseq experiments were also performed with the same samples to compare both platforms. The gene models generated are of high quality, with 98% of the reads retained in the genome, and gene loci mapping. The biological function was inferred by homology searching showing that uniquely shared groups D. magna-D. pulex are the majority (~12K), with fruitfly and Human genes being the minority (~4K). Advance chemometric tools allowed the recognition of several genes that are key for development and in coping with stress provoked by different environmental relevant contaminants. The microarray validation experiment design showed that for most genes, the multiple probes design behave in similar way, thus allowing us to proceed to a refined design (8X6K0X) without losing information at a much more cost-effective fashion. The development of bioinformatic tools specific for Daphnia magna would increase our current knowledge of its molecular regulation and facilitate its adoption as a model organism for regulatory purposes.

79 Toxicogenomic approach to investigate the effects of Cu and Ag (nano)materials in the terrestrial invertebrate E. crypticus: towards the understanding of systems
S.J. Gomes, University of Aveiro / Department of Biology; CESAM; C.P. Roca, Universitat Rovira i Virgili / Substance of Chemical Engineering; J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience; Terrestrial Ecology; M. Amorim, Universidade de Aveiro / Department of Biology and CESAM

High-throughput gene expression tools advance the understanding of mechanisms of toxic-mediated responses. Further, the mechanistic based approach enabled based on omics techniques provides optimization on issues that link changes from higher levels of biological organization. Such data can be integrated e.g. via the Adverse Outcome Pathways (AOPs), towards a systems biology approach. The aim of the present study was to investigate the mechanisms of toxicity for Cu (copper) and Ag (silver) materials using the high-throughput gene expression tool for the soil invertebrate Enchytraeus crypticus (Oligochaeta, Enchytraeidae), a 4x4K custom Agilent microarray. Testing was done linked to reproduction effect concentrations (EC20, EC50) using 3 and 7 days of exposure. The data analysis was performed following standard R/Bioconductor workflows for one-color mRNA microarrays: quality control, background correction, probe selection, normalization and statistical analysis. Besides the normalization methods provided by Bioconductor, we used a novel normalization procedure (vectorial normalization) in order to improve the detection of small changes of gene expression. Results indicated specific mechanisms of responses for the different materials tested. Cu-salt exposure affected mechanisms related with death, homoeostasis and specific mechanisms of the chemosensory system of the enchytraeids. Energetic metabolism was affected differently depending on the copper or silver form. For Ag, results showed that one of the materials caused a more differentiated transcriptomic profile than the others. Commonly across all Ag forms were the effects on cell cycle control associated with impairment of DNA repair mechanism. The study of gene expression pointed differences in gene responses to the various Cu and Ag forms tested, information that is notoriously absent in experiments focusing on the standard effect endpoints survival/reproduction. The Adverse Outcome Pathways (AOP) was important to integrate effects from various levels and provide input for risk assessors.

Keywords: High-throughput, toxicogenomics, mechanisms of response, nanomaterials

80 Comparative genomics in zinc toxicity: a method to bridge current developments
T. De Boer, Vrije Universiteit / Ecological Sciences; R. Vooijs, VU University Amsterdam; J. Legler, VU University / Institute for Environmental Studies; C. van Gestel, VU University Amsterdam / Ecological Science; D. Roelofs, Vrije Universiteit / Inst. of Ecological Science

Two of the most promising developments in the field of environmental risk assessment are Adverse Outcome Pathways (AOP) and Species Sensitivity Distributions (SSD). While one development looks at the effects of chemicals on multiple organismal levels of a single species, the other focuses on the sensitivity to a chemical to multiple organisms. Comparative transcriptomics may be a method to combine these two developments. Comparative transcriptomics (also known as comparative genomics) uses high throughput gene expression analysis such as microarray analysis and RNaseq to compare transcriptomic responses between species and is based on the theory that conserved gene sequences (orthologous genes) between species should act in a more similar way to a stressor then non conserved gene sequences. Here we present a study on the comparative transcriptomic responses to zinc exposure in three different ecological and genomic model species: two terrestrial species (Folsomia candida and Enchytraeus crypticus) and an aquatic species (Daphnia magna). All three species were exposed to 40mg/l ZnSO4 for 48 and 72 hours after which gene expression was measured. Gene expression analysis for single species revealed that a greater effect for the two terrestrial species (in percentage of genes differentially expressed) than for Daphnia magna and the patterns were also different. For Daphnia magna EC50 yielded more significant genes at both days then for the EC10 while in both terrestrial species day 2 exposures yielded more significant genes at the EC10 level than at the EC50 level. Orthoexpression analysis yielded 3268 ortholog groups and 6812 co-ortholog groups. As of now, we only looked at the responses between the two terrestrial species with a comparison for all three species to be completed soon complete. Comparison of gene responses in the ortholog group yielded significant correlations between 303 of the 3268 orthologs. Clustering of these significant orthologs in both species showed that in Folsomia candida the orthologs were more co-regulated then for Enchytraeus crypticus. Comparative transcriptomics/genomics can be a powerful tool to investigate the similarities and differences in species responses when exposed to comparable stressors

81 Network-based pathway analysis improves the interpretation of high-throughput data in toxicology studies
C.P. Roca, Universitat Rovira i Virgili / Department of Chemical Engineering; J. Yang, N. Chatterjee, University of Seoul; J. Choi, School of Environmental Engineering Graduate School of Energy and Environmental system Engineering; F. Giralt, Universitat Rovira i Virgili

High-throughput -omics techniques are powerful experimental tools, able to scan with great detail changes of function in cells. However, using this information to identify physiological and organ responses, as required by the framework of Adverse Outcome Pathways, poses a great challenge. Data analysis tools, such as Gene Enrichment Analysis or Pathway Analysis, address this problem using curated collections of biochemical pathways. Most approaches analyze each pathway independently from the others. This resulting view of the cell is the superposition of all those pathways, without information about how they interact or coordinate to form a single system. We present here a method of pathway analysis which addresses this issue. It is based on a single genome-wide network representation of all the pathways annotated for the target species. We have developed a network statistic that...
generalizes in a simple way the current over-representation analysis. The method conceives the cell as a system of interconnected pathways, instead of a collection of independent biochemical circuits. As a result, it can identify pathway crosstalk and complex multi-pathway effects. Besides explaining the underlying principles of the method, we illustrate the benefits in practice with an study of the toxicity of graphene oxide on C. elegans. Keywords: pathway analysis, networks, high-throughput, systems biology

82 Integration of Proteomic and Metabolomic Profiling for Studying Zinc Oxide Nanotoxicity in Exposed Lung Tissue Cells
T. Kroutil, C. Ranninger, University of Salzburg / Department of Molecular Biology; M. Rehman, University of Tübingen / Center for Bioinformatics Quantitative Biology; R. Reischl, I. Radauer-Preiml, A. Duschl, University of Salzburg / Department of Molecular Biology; O. Kohlbacher, University of Tübingen / Center for Bioinformatics Quantitative Biology; C. Huber, University of Salzburg
This study aimed at the screening of cytotoxic effects of zinc oxide nanoparticles to observe the impact on protein and metabolite abundance and to identify affected biochemical pathways. A comprehensive non-targeted proteomics and metabolomics approach was chosen, which captures the influence of external stimuli on a human alveolar basal epithelial cell line (A549). Six different time points were evaluated (0, 1, 3, 6, 12, 24 h) for three biological replicates comparing controls and nanoparticle-treated cells. Proteins were precipitated, while metabolites were extracted from the cell lysates with cold methanol. For proteome analysis, the alkylated and isotope labeled (TMT six-plex) tryptic peptides were separated by capillary ion-pair reversed-phase HPLC in a 150 x 0.2 mm i.d. monolithic column with followed by linear ion trap-Orbitrap mass spectrometry. Separation of the metabolites was performed with a reversed-phase or HILIC UHPLC system utilizing a polar end-capped C18 phase (Hypersil Gold aQ C18, 100 x 2.1 mm, 1.9 µm) or a hydrophilic stationary phase (Nucleodur HILIC, 150 x 2.0 mm, 1.8 µm). Discovery and identification were based on quadrupole-Orbitrap mass spectrometry. Bioinformatic workflows using the open source software packages OpenMS and KNIME were applied for data evaluation, involving univariate and multivariate statistical approaches to determine differentially expressed proteins and metabolites. In total, 1036 proteins were identified and quantified and 123 of them were up- or down-regulated. Up-regulation was observable for the following proteins: Heme oxygenase (decycling) 1; Histone-lysine N-methyltransferase 2E; sequestosome 1; complement component 1, q subcomponent binding protein and protein tyrosine phosphatase, receptor type, N (HMOX1; KMT2E; SQSTM1; C1QBPF and PTPRN). Twenty-seven molecules are playing a crucial role in the bio function “cellular growth and proliferation”. The proteomic and metabolomic approach is a promising tool for biomarker research of chronic lung diseases. Between 170 and 630 metabolite features were found to be differentially regulated after strict filtering and quality control. The majority of them was down-regulated upon treatment, for example metabolites of the glutathione pathway indicative of oxidative stress. The study clearly shows that both proteomic and metabolomic analysis are valuable tools for studying biological effects of nanoparticles in an epithelial cell model.

Prioritization and management of multiple stressors in river basins: effects of chemical contaminants and natural stressors (II)

83 Assessment of river water quality in catchments: Import of urbanization on particle bound pollutant fluxes
H. Ruenzer, University of Tübingen / Water – Earth System Science; M. Schwientek, Water and Earth System Science; P. Grahwohl, Uni Tübingen / Centre of Applied Geosciences
Transport of many urban pollutants in rivers is coupled to transported of suspended particles, potentially dominated by storm water outflows and mobilization of legacy contamination of sediments. Concentration of these pollutants depends on the mixture of "polluted" urban and “clean” background particles. In the current study, the total concentration of polycyclic aromatic hydrocarbons (PAHs), the amount of total suspended solids (TSS) and turbidity were measured on a monthly basis in water samples from 5 catchments with contrast- ing land use in Southwest Germany over up to 1.5 years. In addition, single flood events with large changes in turbidity were sampled at high temporal resolution. Linear correlations of turbidity and TSS where obtained over all catchments investigated and over an extended turbidity range (up to 2000 NTU for the flood samples). Linear correlations were also obtained for the total amount of PAH and suspended sediment concentrations even for very high turbidity or TSS values (2000 NTU or mg l⁻¹, respectively). From the linear regressions of concentrations of PAHs and suspended particles were obtained – and which varied by catchment. The values comprise a robust measure of the average sediment quality in a river network and may be correlated to the degree of urbanization represented by the number of inhabitants per total flux of suspended particles. The findings are promising for other particle-bound contaminant fluxes (PCBs, phosphorus, and several heavy metals, etc.). Recent investigation focus on the type and geochemistry of suspended particles representing different time periods of pronounced events (total organic carbon and carbonate content, grain size). 1st results showed that concentrations of PAHs on suspended particles did not vary much in different grain size fractions.

84 Contribution and remobilization of semi- volatile organic contaminants (SVOCs) from a melting Himalayan glacier
B.M. Sharma, TERI University / Department of Natural Resources; L. Nizzetto, NIVA; G.K. Bharat, S. Tayal, The Energy and Resources Institute (TERI); L. Melymuk, Masaryk University, Faculty of Science, RECETOX / RECETOX Research Centre for Toxic Compounds in the Environment; O. Säfka, Research Centre for Toxic Compounds in the Environment RECETOX; P. Pribylova, Masaryk University; O. Audy, Research Centre for Toxic Compounds in the Environment RECETOX; T. Larsen, Norwegian Institute for Water Research NIVA
In the past decades, geological environment of the Himalayan mountain glaciers have experiencing the potential impacts of “climate change”, and there are evidences that it may influence the remobilization of semi-volatile organic contaminants (SVOCs) and similar toxic pollutants on a regional scale. In the present study, we have observed the emission of dichlorodiphenyltrichloroethane (DDTs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) in the meltwater of a large Himalayan glacier and have also assessed their relevance on regional scale. A simultaneous sampling of atmospheric meltwater, and bulk deposition was performed during the months of April – September 2013 along the course of the river Gangan of India, which was, included for or originating from the Himalayas. We observed a net deposition of DDT and three and four ringed lighter PAHs to the meltwater by gas exchange. In contrast, PCBs volatilize to air, implying glacier meltwater behaving as an important secondary source of PCBs. In the case of four and five ringed heavy PAHs, contribution from glacier was assessed as an offset between their total discharge in the meltwater and bulk atmospheric inputs at two possible nearest control points to glacier snout. The results elucidate that the glacier meltwater is a powerful source of highly toxic four and five ringed heavy PAHs, which were historically accumulated over the glacier from deposition of contaminated atmospheric particles. On a regional scale, the contribution of meltwater associated SVOCs to the total discharge at downstream control points seems significant, thereby indicating glacier as a highly probable secondary source.

85 Risk Assessment of stream water: linking mass discharge from contaminated sites in groundwater with water health
B. Møller, Aarhus University / bioscience; S. Höss, Ecossa; W. Transupper, University of Bielefeld; U.S. McKnight, Technical University of Denmark (DTU) / Environmental Engineering; P.L. Bjerg, Technical University of Denmark DTU / Environmental Engineering
Streams are impacted by significant contamination at the catchment scale, in particular they are often affected by multiple stressors, including xenobiotic compounds in groundwater originating from contaminated sites, diffuse source pollution (e.g. surface run-off, tile drains) and water abstraction. With the enactment of the European Water Framework Directive (WFD) in 2000 and the corresponding Groundwater Daughter Directive (GDD) in 2006, all Member States are required to ensure the good chemical and ecological status of groundwater (GW) and water abstraction sites in groundwater (SW) and pore water (P). The chemical quality of surface water is defined in part by a set of environmental quality standards (EQS) on priority substances (EQS Directive (2008/105/EC) and in part by the individual Member States (e.g. Danish EPA, 2010, BEK nr. 1022). However the relationships between the quality elements used for the classification of ecological status (biological, hydromorphological and physicochemical) is defined in a more holistic manner leaving the Member States free to define the details of their own assessment tools. In this study we evaluate biological methods/tools for risk assessing the impact of xenobiotic contaminated groundwater entering a stream. We focus specifically on the use of invertebrate fauna for quantifying the link between chemical and ecological status. Our results show the presence of both a local and downstream impact resulting from the contaminated groundwater entering the Grindsted stream for both the meio- and macrobenthic fauna. This indicates that this type of stressor affecting water bodies are of a significant importance and must therefore be included when conducting risk assessments of contaminated sites. The toxic potential (TU$_{max}$) of the identified xenobiotic contaminants was generally very low suggesting that long term exposure may generate un expected strong effects in the field. The best estimate for toxicological significance of ecological status (species richness and diversity) in the stream based on the chemical data, was achieved by calculating TU for all three phases (SW, GW and pore water) from each of the investigated locations in order to assess whether the ecological status was affected.

86 Contaminants of Emerging Concern in Fish from 4 Spanish Mediterranean Rivers
Y. Pico, University of Valencia / Medicine Preventive; V. Belenguer, University of Valencia; C. Corellas, IDAEA / Department of Environmental Chemistry; M.
Mediterranean Rivers are ecologically vulnerable with prominent losses in biodiversity caused among other factors by pollution. However, the pollution status and effects of emerging contaminants is still largely unknown because the data on these contaminants is not frequently available or the concentrations of particular contaminants in water. The purpose of this study was to provide an accurate spatial analysis of emerging contaminants in fish from the most important Spanish Mediterranean Rivers (Guadalquivir, Ebre, Llobregat and Jucar), so comprehending regional differences in contaminant burdens, and comparing contaminant concentrations to human and wildlife health thresholds to better understand potential effects in these areas. Concentrations of emerging contaminants in 14 fish species were determined. Among ECDs, BPA was at high concentration in wild fish (up to 223.91 ng/g in Llobregat river), whereas TBP, caffeine and methyl and benzylophenan were the pharmaceuticals detected at concentrations up to 10 ng/g. The most ubiquitous and recurring compound was diclofenac. The UV filters were much more frequent in fish from Guadalquivir river basin (up to 80 % of the samples) than in those from Llobregat (91 %), Ebro (18 %) or Júcar (11 %). Pesticides and herbicides were mostly detected on the Jucar River where atrazine desisopropyl, azinphos ethyl, carbofuran, chlorpyriphos, diazinon, dimethoate, ethion, hexythiazox, imazalil, methoalachlo and omethoate were identified. The highest PBDE and PFCs concentrations were in the Llobregat river basin (55.9-520 ng/g lw.). There was a good relationship between the presence of these contaminants in water and in sediments, and in fish. However, not all the contaminants detected in water samples were also in fish. A better relationship can be established between compounds detected in fish and in sediments. The levels and concentrations of contaminants, as well as the co-occurrence of different classes of them in fishes, are clearly related with the land uses of the area. Contaminant monitoring efforts should continue and management actions can be taken for the protection of aquatic ecosystems. Acknowledgement The authors thank the Spanish Ministry of Economy and Competitiveness for the financial support through the project SCARCE (Consolider-Ingenio 2010 CSD2009-00065).

87 High contribution of legacy pesticides to predicted water and sediment toxicity in agricultural streams
J.J. Rasmussen, Aarhus University / biocience; P. Wiberg-Larsen, Aarhus University DMU; A. Baatrup-Pedersen, Aarhus University DMU / Department of Biological Sciences; D. Olesen, University of Copenhagen; N. Friberg, NERI (DMU) / Freshwater Ecology
We revealed a history of legacy pesticides in water and sediment samples from 19 small streams across an agricultural landscape. Dominant legacy compounds included organochlorine pesticides, such as DDT and Lindane, and the organophosphate pesticide Chlorpyrifos which has long been banned in EU. High concentrations of pesticides during catching events with a large proportion of arable farmland suggesting that they originated from past agricultural applications. These stream samples also revealed the highest concentrations of registered pesticides that are currently in use when compared with samples from less agricultural impacted streams. Concentrations of legacy pesticides alone exceeded the safety thresholds for daphnia in 6 water samples and they increased sum of toxic units (SumTU_{most}) significantly in storm water samples from agricultural impacted streams. These streams also exceeded safety thresholds in 50 % of the sediment and suspended sediment samples and the average contribution of legacy pesticides to SumTU_{most} for bed sediments and suspended sediments was > 90%. Our results suggest that legacy pesticides can be highly significant contributors to the contemporary toxic exposure of stream biota, especially macroinvertebrate communities, and that those communities were primarily exposed to legacy pesticides via the sediment.

88 Out of sight out of mind: A case study of the long term effect of capping a sea depot of hazardous waste
K.S. Hatlen, Uni Research, SAMARIN / Environment; M. Haave, Uni Research, SAMARIN / Uni Research Environment
Kollebøgen bay was for 45 years a depot for Bergen citizens, accumulating sand and gravel in 2005. Kollebøgen is now utilized as a recreational area for musse, bathers and naturalists. Yearly environmental monitoring has been conducted from 2004 till present, comprising sampling of bottom sediment, blue mussel (Mytilus edulis), liver and filet of cod (Gadus morhua) and filet of turbot (Scophthalmus maximus). The samples have been analysed for PCBs (ICES 7) in sediment and biota, dioxin like (dl) PCBs in filet and liver of cod and PAH-16 and heavy metals in sediment. Because the area is used by people, sand on the beach was sampled for PCBs (ICES 7), and dietary advice for sea food consumption is given each year. Levels of PCBs in bottom sediment increased after 2008 at all stations. In 2012, the sediment at Kolle 1 and Kolle 5 contained 150,4 and 92,8 mg/kg (DW), respectively. Sediment on the beach did however not contain elevated levels of PCBs. The bottom sediment was cleaned in 2009 by a company (Classification by Norwegian Environment Agency) for heavy metals and TBT at Kolle 1, 2 and 5. PCBs in blue mussel had a steady decrease from 2004 (av:10,4 µg/g) to 2013 (av:3,9 µg/kg). A decrease of PCBs is also seen in filet of cod, while the levels were high in cod liver in 2009 and 2010 and in turbot filet in 2009 and 2012. The levels of dl-PCBs lay above threshold values (WHO-TEF 2005) for cod liver in 2009 (> 752 pg/g) and turbot filet in 2009 (43,30 pg/g TEFQ) and has increased since 2012. The increased concentration of environmental contamination after 2008 seen in many of the parameters, was explained by a ROV survey in 2013. A crevasse in the cover of approx. 100m was detected, rendering contaminants available to the environment. Hence the results have uncovered that attempts to mend a severe pollution problem in a recreational area, was successful for a few years only, and needs a long term solution.

Metals in the Environment: Fate, Specification and Bioavailability in Water, Soil and Sediment (II)

89 A comparison between Diffusive Milli-gels and Chelex column assessment copper bioavailability and toxicity to Ceriodaphnia dubia
M. Perez, LCaBIcEpRM; LcBie; S.L. Simpson, CSIRO Land and Water / Centre for Environmental Contaminants Research; S. Reynaud, G. Lespes, M. Potin-Gautier, NIST/University of Pau; E. Mignard, SOLVAY; P. Chery, Université Bordeaux; J. King, CSIRO Land and Water; C. Jarolimov, M.S. Adams, CSIRO, D. Schaumloeffel, B. Grassl, NIST/University of Pau
Environmental risk management requires profound knowledge of the uptake of contaminants by biological systems. Copper is an essential trace element for living organisms but can become toxic above a certain concentration. The strong complexation of copper by a naturally occurring organic ligands, e.g. humic and fulvic acids, lowers the bioavailability of copper and the rate of uptake by organisms. Therefore, the determination of the bioavailability of copper is essential for environmental management. Based on the concept of diffusive gradients in thin films (DGT), but with differing geometry, the Diffusive Milli-gel (DMG) passive sampler was developed for measuring dissolved copper concentrations in natural waters. DMG are composed of a spherical polyacrylamide gel beads in which a copper exchanger is incorporated: Chelex 100. The manufacturing technique uses a micellific procedure that allows precise control of the size and the geometry of DMG beads of around 1 millimeter diameter. The main advantage of this system is to increase the exchange surface and allows a copper diffusion in three dimensions. The DMG technique was applied to assessing the bioavailability of copper in natural waters through comparison with a well-established Chelex-column method and bioassays with Ceriodaphnia dubia. The study used ten different waters, with a range of dissolved organic carbon (DOC), hardness and alkalinity, that were spiked and equilibrated with copper. The labile copper concentrations measured by the DMG and Chelex-column methods and toxicity response was most strongly influenced by DOC. A strong linear relationship between DOC and the Five-second lethality concentration (LC50) for Ceriodaphnia dubia was observed (r² = 0.981). Water hardness and alkalinity did not significantly influence the results. The results indicated that the DMG may provide a similar ability to Chelex-column based approaches to predicting influence of DOC on the bioavailability and toxicity of copper in natural waters. However, the DMG methods can be more easily applied in situ and provide a time-integrated measurement of labile copper, while remaining a good predictor of the toxicity of copper to aquatic organisms.

The nickel BLMs applied within Europe show some shortcomings in the prediction of nickel toxicity to Australian test species, although to a large extent this is likely to be due to the relatively large apparent differences in the hardness of waters found in Europe and Australia identified by the water chemistry survey. The lower variation limit of the European ChemPLM (Bioavailability of 2 mg/L), and potentially excludes a relatively large proportion of some types of Australian waters from implementation of the existing nickel BLMs. The D. magna BLM was able to make reliable predictions of nickel toxicity to the tested...
invertebrates (Ceriodaphnia and Hydra) when the low hardness Magela Creek water was treated as an outlier due to its low water hardness. Poor predictions were made for the toxicity of nickel to fish in Pechelbarra water, and for the toxicity to Chlorella in Tea Tree water. The C. dubia BLM provided the most accurate predictions for nickel toxicity to Lemma. Modifications to either the fraction of active fulvic acid, or the binding parameters for nickel to fulvic acid, had only a relatively limited effect in improving predictions. Increasing the binding constants of both calcium and magnesium to the biotic ligand provided very good predictions for fish, Ceriodaphnia, Hydra, and Chlorella in all of the tested waters, but did not improve predictions for Lemma. This approach does not require any need to distinguish between extremely soft (e.g. Magela Creek < 2 mg l⁻¹ CaCO₃) and soft (e.g. Wellington 58 mg l⁻¹ CaCO₃) waters when making BLM predictions. It is likely that the approach will perform equally well for calcium and magnesium which is observed to the previously tested North American waters is due to the low concentrations of calcium and magnesium found in some Australian waters. There are indications that soft-water organisms respond differently to hard-water organisms regardless of their origins.

91 Nickel in lotic sediments: Understanding the role of Fe oxides in oxidic surface sediments
D. Costello, Kent State University / Biological Sciences; R. Mendonca, Universidade Federal de Pernambuco / Depto. de Zoologia, Centro de Ciências Biológicas; A. Harrison, University of Michigan / School of Natural Resources Environment; M. Dally, University of Michigan / School of Natural Resources Environment; C.E. Schlekat, NiPERA; E.R. Garman, NiPERA / Ecotoxicologist; C.R. Hammerschmidt, Wright State University / Department of Earth Environmental Sciences; A. Burton, University of Michigan / School of Natural Resources Environment
The toxicity of Ni in sediments is modified by the availability and cycling of elements, such as iron and manganese complexes that can limit Ni bioavailability. Under anoxic conditions, reduced sulfur (e.g., AVS) and organic carbon (OC) are the primary binding fractions for Ni; however, under oxidic conditions, AVS is unstable and OC can be rapidly respired. Fe oxide minerals are stable in oxidic sediment and can sorb Ni. Currently, environmental quality standards (EQS) for Ni in sediment recognize that AVS and OC can reduce Ni toxicity, but these EQS may not be appropriate for oxidic sediments. We completed a laboratory experiment where two reference sediments with differing binding capacities (i.e., AVS, OC, Fe) were spiked with Ni (reference +4 concentrations) and equilibrated under anoxic conditions. These sediments were aged in a flow-through mesocosm (100 days) while concurrently exposing Hyalella azteca to the sediments to measure changes in toxicity during aging. Concurrent sampling of sediment geochemistry revealed dynamics in chemistry and toxicity as surface sediments oxidized. Total Ni pools remained stable but porewater Ni declined, which suggests a change in Ni speciation as sediments aged and oxidized. Further, our toxicity tests showed that sediments became less toxic as they aged. Fe oxides increased through time in surface sediments and sorbed a large fraction of Ni (50-80%). Additionally, a field experiment was conducted in four tributaries of the South River (TPM, MB, Canada): two tributaries impacted by mine effluent and two reference sites. At each tributary we assessed acute toxicity of overlying water and sediment with in situ chambers (H. azteca and Lymnaea stagnalis), native benthic macroinvertebrate community, overlying water chemistry, and surface and deep sediment chemistry. Effluent tributaries contained elevated sediment Ni (up to ~500 mg/kg) and higher AVS concentrations in effluent (than reference sites). Similar to laboratory studies, we observed Ni sorbed to Fe oxide minerals at effluent sites. In situ toxicity assays revealed that neither overlying water nor sediments in the effluent streams were acutely toxic. Sensitive macroinvertebrates (e.g., Hexagenia) were observed in reference and effluent tributaries, which suggests that sediment Ni is not causing impairment. Collectively, these studies demonstrate that current sediments Ni EQS is conservative and Fe oxides should be considered in future Ni bioavailability models.

92 The TICKET-UWM model can improve metals/inorganics environmental fate modelling for management
E. Verdouten, ARCHE; H. Waetser schoot, Eirometaux; K.J. Rader, Mutch Associates, LLC; K. Oorts, ARCHE; K. Delbeke, European Copper Institute; B.A. Stubblefield, Oregon State University / Environmental and Molecular Toxicology; P. Van Sprang, ARCHE
EUSES is a multimedia fugacity based fate model used as a decision-support instrument in the evaluation of the TH and VME conditions. This model, which enables the user to calculate the risk for man and environment at the local, regional and continental scale, has been developed primarily for organic substances and needs refinements to predict the fate and exposure of metals. The Tableau Input Coupled Kinetic Equilibrium Transport - Unit World Model (TICKET-UWM) was therefore explicitly developed to model transport, fate, and toxicity of metals and considers additionally key processes such as precipitation by aqutic inorganic and organic ligands (e.g., DOC), adsorption to particulate organic carbon (POC), binding to biological receptors (biotic ligands), and transport of dissolved metals and solids between the water column and sediment. The aim of this work is to present the conceptual differences between both multi-media fate models for use in metal risk assessments. Additionally, a comparative model simulation using realistic examples for the metals Cu and Co shows clear differences in exposure concentrations when metal specific features are considered. The TICKET-UWM model allows for both risk calculations. As a general conclusion it is therefore strongly advocated to implement metal-specific features in decision-support models such as EUSES in order to increase the ecological relevancy of the risk assessment for metals. The risk characterisation part allows to use a high quality reference database for the toxicity of the metals included in the model. A comparison between EUSES and TICKET-UWM clearly illustrates that metal-specific approaches significantly affect the outcome of an exposure and risk assessment. It is therefore strongly advocated to further investigate those differences in detail and implement metal-specific features in decision-support models such as EUSES in order to increase the ecological relevancy of the risk assessment for metals.

93 How do aquatic organisms handle non-essential and potentially toxic metals in their intracellular environment? A comparison among different metals and across different species
P. Van Sprang, Université du Québec, INRS / INRS Eau Terre Environnement; P. Couture, INRS / Centre Eau Terre Environnement; C. Fortin, University of Quebec / Centre Eau Terre Environnement; L. Hare, INRS-ETE; M. Rosabal, INRS-ETE / Centre Eau Terre Environnement
Over the past 10+ years, we have investigated how aquatic organisms cope when exposed to non-essential metals such as silver, cadmium, nickel and lead. These elements cause significant toxicity in aquatic species, and invertebrates (e.g., Hydra or Lymnaea stagnalis) but for a given metal they also differ, which implies that their handling depends on the element. The toxicity of Ni in sediments is modified by the availability and cycling of elements, such as iron and manganese complexes that can limit Ni bioavailability. Under anoxic conditions, reduced sulfur (e.g., AVS) and organic carbon (OC) are the primary binding fractions for Ni; however, under oxidic conditions, AVS is unstable and OC can be rapidly respired. Fe oxide minerals are stable in oxidic sediment and can sorb Ni. Currently, environmental quality standards (EQS) for Ni in sediment recognize that AVS and OC can reduce Ni toxicity, but these EQS may not be appropriate for oxidic sediments. We completed a laboratory experiment where two reference sediments with differing binding capacities (i.e., AVS, OC, Fe) were spiked with Ni (reference +4 concentrations) and equilibrated under anoxic conditions. These sediments were aged in a flow-through mesocosm (100 days) while concurrently exposing Hyalella azteca to the sediments to measure changes in toxicity during aging. Concurrent sampling of sediment geochemistry revealed dynamics in chemistry and toxicity as surface sediments oxidized. Total Ni pools remained stable but porewater Ni declined, which suggests a change in Ni speciation as sediments aged and oxidized. Further, our toxicity tests showed that sediments became less toxic as they aged. Fe oxides increased through time in surface sediments and sorbed a large fraction of Ni (50-80%). Additionally, a field experiment was conducted in four tributaries of the South River (TPM, MB, Canada): two tributaries impacted by mine effluent and two reference sites. At each tributary we assessed acute toxicity of overlying water and sediment with in situ chambers (H. azteca and Lymnaea stagnalis), native benthic macroinvertebrate community, overlying water chemistry, and surface and deep sediment chemistry. Effluent tributaries contained elevated sediment Ni (up to ~500 mg/kg) and higher AVS concentrations in effluent (than reference sites). Similar to laboratory studies, we observed Ni sorbed to Fe oxide minerals at effluent sites. In situ toxicity assays revealed that neither overlying water nor sediments in the effluent streams were acutely toxic. Sensitive macroinvertebrates (e.g., Hexagenia) were observed in reference and effluent tributaries, which suggests that sediment Ni is not causing impairment. Collectively, these studies demonstrate that current sediments Ni EQS is conservative and Fe oxides should be considered in future Ni bioavailability models.

94 Radioisotope techniques and aquatic ecotoxicology: understanding kinetics and organ distribution of metals
T. Cresswell, ANSTO Institute for Environmental Research; S.L. Simpson, CSIRO Land and Water / Centre for Environmental Contaminants Research; D. Mazumder, Australian Nuclear Science and Technology Organisation / Institute for Environmental Research, P. Callaghan, A. Nguyen, Australian Nuclear Science and Technology Organisation / Life Sciences; M. Corry, Australian Nuclear Science and Technology Organisation / Institute for Environmental Research; M. Rosabal, INRS-ETE / Centre Eau Terre Environnement
Gamma-emitting metal radioisotopes are valuable tools for studying metal bioaccumulation in aquatic systems, allowing exposure or consumption of multiple metals to be analysed rapidly, at multiple intervals during an exposure period, without sacrificing the organism. Furthermore, autoradiography of cytosedimented organisms enables the organ distribution of accumulated metals to be visualised and quantified. In the 1st study, Macrobachium australis (prawns) were exposed to dissolved ⁶⁵Cd and ⁹⁰Zn either individually or as a mixture of both metals for 3 weeks before being transferred to a reference free water for 2 weeks. At the end of the depuration period, prawns were sacrificed, embedded in a resin and snap frozen in liquid N₂ prior to autoradiography. The 2nd study exposed one set of 15 prawns to dissolved ⁶⁵Cd for up to 2 weeks, with three prawns being removed for autoradiography at set times to determine the location of Cd accumulation in organ tissues. A second set of 30 prawns was exposed to dissolved ⁶⁵Cd for either 6 hours (acute exposure) or 7 days (pseudo chronic exposure) and sacrificed to depurate for up to 3 weeks. Prawns were removed from the depuration tanks at set times for autoradiography. For the 1st study, the majority of Cd uptake was localised within the gills and hepatopancreas, while Zn accumulated in the antennal gland at concentrations orders of magnitude greater than in other organs. This suggested that
Emerging contaminants of urban origin: sources, sampling, analytical issues and remediation techniques (II)

95 Spatial Variability of Flame Retardants in Indoor Dust
S. Jilková, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; L. Melnyuk, Masaryk University, Faculty of Science, RECETOX / RECETOX Research Centre for Toxic Compounds in the Environment; J. Vojta, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; P. Bohlin Nizzetto, NILU Norwegian Institute for Air Research; M. Knīć, J. Klamova, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment

Indoor dust is often used to evaluate levels of semivolatile organic compounds indoors, particularly for those with indoor sources, such as flame retardants (FRs). Yet there are uncertainties about the type of information that can be obtained from indoor dust. We conducted detailed sampling to assess spatial variability in indoor dust concentrations and surface loadings, and the relationship between FR sources and dust. From this, we assess the utility of dust for source identification, evaluating overall indoor levels, or human exposure, and identify what practices in dust collection must be used to achieve these ends. In a large residential room 18 individual samples were collected, covering a range of surfaces (carpet, hardwood floor, furniture and electronic surfaces, and windows). Samples were analyzed for 12 PBDEs, and 18 novel halogenated flame retardants (NFRs). Concentrations of NFRs were dominated by BEH-TEP, syn- and anti-DDC-Cl and I- and so-DBE-DBCH. NFRs were 75.9-884 ng/g, and surface loadings were 0.807-685 ng/m². The spatial distribution of BDE-209 in indoor dust was consistently higher than on elevated surfaces (e.g. furniture, tables, windows), with BDE-209 making up 83-97% of PBDEs on floors, while on surfaces 87-97%. Of the NFRs, 8 were below detection in all samples (TBP-BAC, TBB, TBP-DBPE, TBCO, TBCT and PBBA). Concentrations of TBBPAs were measured in undiluted dust samples.

96 Bioaccessibility of flame retardants in household dust
C.D. Collins, Reading University / Soil Research Centre; S. Aleaga-Garcia, University of Reading

Flame retardants (FRs) are frequently added to a wide range of household products e.g. furniture, electrical goods. Many of these products are known to be toxic and significant quantities have been reported in household dust. There is therefore the potential for FRs to be ingested by children, who can be exposed via ingestion. To fine tune risk assessments bioaccessibility measures of these dusts can be undertaken to determine the likely total amount of FRs released in the gastro intestinal tract. Using an established bioaccessibility test for organic pollutants (CEPBEAT) a range of FRs were studied. The principal control on the release of the FRs from the dust was the log KOW of the parent compound. The numerical study also showed a significant increase in FR bioaccessibility when it was operated in the fed state. Using a level 1 fugacity model the results were well predicted suggesting a model approach may be appropriate for a broader suite of compounds.

97 Air contamination by endocrine disruptors: comparison of active and passive samplers coupled to a multi-residue method
E. Moreau-Guigné, EPHE; F. Alliot, ecole pratique des hautes études UMR SISYPHE

Chevreul, ecole pratique des haute études UMR SISYPHE

Over 2 years, spatial (urban/suburban/forest area) and time scale (seasonal/inter-annual) variations of a wide variety of endocrine disruptor compounds (EDCs n = 59) were investigated in ambient air. Associated to a multi-residue analytical method, three different samplers were used: an active sampler and two passive samplers, PUF and leaves of plane trees (Platanus hispanica). It was also the first investigation of air contamination at a regional scale in France, which considered EDC distribution in gaseous and particulate phases. All EDCs except tetrabromobisphenol A were encountered in the ambient air at different frequencies up to 100%. Phthalates were the most abundant with concentrations ranging from 10 to 100 ng.m⁻³, followed by polycyclic aromatic hydrocarbons (PAHs) about 1 ng.m⁻³. Polychlorinated biphenyls (PCBs) and bisphenol A concentrations were much lower (around 0.1 ng.m⁻³). In spite of their relatively low vapor pressures, most of EDCs were mainly distributed in the gaseous phase (> 80% in summer) which displayed a high scattering that might explain EDC ubiquity even far away from their emission sources. EDCs were more abundant in summer and rather governed by volatilization, except PAHs which emissions were higher in cold season linked to combustion processes. Bisphenol A concentrations were correlated to particle matter < 10 µm (PM10) while PCBs and diethylphthalate concentrations were correlated to the temperature. Molecular profiles in PUF samples were similar to those observed in active samples and air concentrations were of the same order. For pollutants trapped inside plane leaves, the first results concern PAHs and show the same profile between PUF and active samples. However, the number of molecules trapped by leaves was higher than in air. For both samplers, the position of the cuticular organs in the tree is influenced. The quantity of pollutant trapped in leaves is also correlated to the air concentration close to the tree. Results for PCBs, PBDEs and phthalates seem to confirm the relationship between leaf and air contaminations. It appears that plane leaves are a good indicator of air quality.

98 Photodegradation of widely used antibiotics - Real world vs laboratory studies. Are algal ponds the way forward?
F. Tzelepí, Lancaster University / Centre for Global Ecoinnovation; C.J. Hallsal, Lancaster University / Lancaster Environment Centre; K. Waterhouse, Bowland Analytical Support Ltd.

There are a growing number of studies that have detected pharmaceuticals and their active metabolites in a variety of environmental waters including sewage effluent, surface waters, groundwater and even drinking water. However, there are knowledge gaps regarding the fate of these chemicals and how they breakdown under different environmental conditions, perhaps giving rise to recalcitrant degradation products. A series of experiments using field and laboratory based photolytic degradation studies were conducted under a simulated-solar light source for a number of common veterinary antibiotics. Rates of degradation were related to the light dose alongside the qualification of key degradates. These degradation tests were then repeated under natural sunlight in a range of different water types including filtered and unfiltered lake water. Some notable differences were apparent. Interestingly, the presence of microorganisms in the water, particularly certain algal species, appears to be increasing the rates of photochemical degradation for a few of the test chemicals, including degradation of some of the key transformation products. The work here suggests that novel, low-technology methods, based on culturing key aquatic microorganisms, may serve as a plausible route for enhanced removal of dissolved antibiotics and other pharmaceuticals from sunlit waters.

99 Reactivity of selected pharmaceuticals with ozone and their fate in advanced wastewater treatment
E. Bosznovics, Silesian University of Technology / Department of Environmental Biotechnology; M. Bourgin, Eawag Swiss Federal Institute of Aquatic Science and Technology; M. Bohrer, Eawag / Department of Process Engineering; H. Siegrist, Eawag / Process Engineering; J. Hollender, Eawag / Environmental Chemistry; C.S. McArdell, Eawag / Department of Environmental Chemistry; U. von Gunten, Eawag Swiss federal Institute of Aquatic Science and Technology

In the recent years the presence of anthropogenic contaminants in the aqueous environment has gained increasing concern. Because of the low removal efficiency of conventional municipal wastewater treatment for many polar contaminants like pharmaceuticals, new treatment methods have been developed. Ozone-based processes have been investigated in laboratory and also pilot-and few full-scale plants. The results showed the huge potential of these methods. The aim of the study was to investigate advanced methods using ozone for wastewater treatment. In the scope of the study was: (i) determination of reactivity of compounds in reaction of O₃, (ii) identification of ozonation products (OPs) of highly reactive compounds, (iii) investigation of the fate of micropollutants in the ozonation process performed at the full scale ozone facility in the wastewater treatment plant (WWTP) Neuburg in Dübendorf. The Neuburg WWTP is the first facility in Switzerland with such a permanent equipment. In the study 8 pharmaceuticals which occur in significant concentrations in WWTP effluents are investigated- 2 antihistamines drugs (cetirizine and fexofenadine), an antipsychotic drug (amisulpride) and 5 hypertension drugs from sartans family (candesartan, losartan, telmisartan, irbesartan, eprosartan). Investigated compounds represent
wide range of reactivity, including very reactive compounds such as cetirizine (kO3 = 1.7·10^7 M^-1 s^-1), medium reactive compounds such as candesartan (kO3 = 563 M^-1 s^-1), but also compounds with very low reactivity such as ibesartan (kO3 = 23 M^-1 s^-1). Laboratory studies proved that ozonation leads to formation of transformation products rather than full mineralization. For instance for cetirizine list of 10 OPs was proposed, and 3 of these compounds have a commercial standard. These OPs were included into the monitoring list in Neugut WWTP. Preliminary studies conducted in the full-scale ozonation facility at Neugut WWTP validated the results obtained in the lab-scale. Compounds considered as highly reactive were removed efficiently during ozonation, while the compounds of low reactivity were still detected at lower concentration in the effluent. In parallel to the degradation of parents compounds, the formation of OPs was observed, for instance cetirizine acetyl derivative while cetirizine was degraded. Detailed studies concerning the fate of organic micropollutants in advanced treatment of wastewater with ozone are in progress.

100 Performance assessment of advanced water treatment processes for the removal of per- and polyfluoroalkyl substances (PFASs)

L. Ahrens, Swedish University of Agricultural Sciences (SLU) / Dept of Aquatic Sciences and Assessment; S. Lundgren, S. England, C. Persson, Swedish University of Agricultural Sciences SLU / Department of Aquatic Sciences and Assessment; P. McCleaf, Uppsala Vatten; E. Lavonen, A. Keucken, Swedish University of Agricultural Sciences SLU / Department of Aquatic Sciences and Assessment; P. Ericsson, Norrvatten; S.J. Kohler, Swedish University of Agricultural Science / Department of Aquatic Sciences and Assessment; K. Wiberg, Swedish University of Agricultural Sciences (SLU) / Department of Aquatic Sciences and Assessment

Per- and polyfluoroalkyl substances (PFASs) comprise a diverse group of chemicals that have been widely used as processing additives during fluoropolymer production and as surfactants in consumer applications for over 50 years. PFASs are persistent against typical environmental degradation processes and have been found ubiquitously in water, air, food, wildlife and humans. Recently, several drinking water sources had to be restricted or even shut down because of chemical pollution from PFASs in Stockholm and Uppsala, Sweden. In this study, the removal efficiency of PFASs will be evaluated using lab-scale, pilot-scale, and full-scale water treatment techniques. In addition, the effect of the character of dissolved organic carbon (DOC) on the removal efficiency of PFASs will be evaluated. The results showed the best removal efficiency using nanofiltration (NF) membrane techniques (in average 50%), followed by magnetic ion-exchange resin (MIEX®, 33%), iron (III) chloride (FeCl₃), 16%, and 20 mg L⁻¹ powdered activated carbon (PAC, 14%). Different removal efficiencies were found for waters having widely different organic carbon compositions, indicating that the DOC characteristics (e.g. source, relative age and degree of humification, pH and absorbance) have an influence on the removal efficiency of PFASs in water.

Overall, the results of this study will improve our understanding of the removal efficiency of advanced water treatment processes for PFASs and the role of DOC. The study results will help the Swedish drinking water industry to make the right investments in future drinking water processes.

102 Environmental occurrence and exposure of 182 POPs and pseudo-POPS in surface water in France

E. Botta, INERIS; H. Budzinski, University of Bordeaux / UMR EPOC Equipe LPTC; F. Lezotrenn, S. ANDRES, INERIS; F. Alliot, M. Chevreuil, ecole pratique des hautes études UMR SISYPHE; M. Devier, University of Bordeaux / UMR CNRS EPOCLPTC; P. Labadie, University of Bordeaux / UMR EPOC Equipe LPTC; C. Cren, Institut des Sciences Analytiques UMR TRACES Team; e. vuillet, ISA; D. Amoux, LCABIE IPREM / UMR CNRS; e. tesser, Université Paris; M. Montperrier, Université de Pau; V. Dulio, INERIS

A screening study of emerging contaminants was carried out in 2012 in surface waters in both metropolitan France and overseas departments (Martinique, Guadeloupe, Réunion, Mayotte and French Guiana) as part of the National Action Plan against pollution of the aquatic environment, which implies the regular updating of the lists of substances to be included in monitoring programs. Different data were collected, such as the presence/absence of each investigated compound (concentration levels below the limit of detection) of concentration observed in the aquatic environment and the degree of exceedance of predicted no effect concentration thresholds (PNEC - Predicted No-Effect Concentrations). 182 substances were selected (out of which 82 analytes to be measured in the water matrix, 134 in the sediment matrix and 48 in both matrices). The substances monitored in this campaign included a large range of contaminants (PAHs & degradation products, alkyl perfluorinated compounds, plasticisers, pharmaceuticals, pesticides, antidepressants, petrol additives, industrial products and personal care products). A total of about 400 monitoring data were collected for each substance for the water matrix and 150 for the sediment matrix. In conclusion, this screening study allowed the collection of about 50000 datasets for chemical and biological analysis of surface waters in continental France and Guadeloupe, Réunion, Mayotte and French Guiana as part of the National Action Plan against pollution of the aquatic environment.

103 Occurrence of emerging contaminants in environmental compartments in relation to substance properties - a case study based on Swedish and Nordic screening studies

E. Brorström-Lundén, IVL Swedish Environmental Research Institute; M. Rahmberg, IVL Swedish Environmental Research Institute; J. Munthe, IVL Swedish Environmental Research Institute Ltd

In order to identify and investigate the presence of emerging chemicals in the Swedish environment, screening studies have been carried out for more than 10 years. Screening in this context means that environmental samples from various matrices in different geographical locations, also including remote areas, are analysed for certain selected chemicals. The overall objective of a screening study is to investigate the occurrence and concentration levels of selected chemicals in a variety of environmental media. Additional aims are to identify potential pathways that highlight important transport pathways and to investigate possible up take in biota and accumulation in the ecosystem and risk for human exposure. How widespread a chemical is in the environment depends on factors such as emission sources, use pattern and on its physicochemical properties, e.g. persistence. Screening is a first step in procedures for risk estimates, prioritization procedures and control measures. It will give an overview of the occurrence of emerging chemicals in different environmental compartments by sorting and ranging data in relation to substance properties using multivariate methods such as Principal Component Analysis (PCA) to determine if any patterns and/or co-variation exist in the data. In this case study, environmental pathways and risks will be identified using this methodology. Examples of chemicals which have been included in screening studies are such as flame retardants (brominated phosphors), plasticizers (phthalates), pharmaceuticals and chemicals included in personal care products (siloxanes, UV-filters). Several of the screened substances mentioned above are frequently present in the Swedish environment and in most of the included matrices.
Occurrence, distribution and bioaccumulation of persistent priority and emerging contaminants in coastal settings (Bay of Cadiz, SW Spain)

M.G. Pintado-Herrera, University of Cadiz; E. Gonzalez-Mazo, University of Cadiz / Physical Chemistry; P. Luna-Martín, Physical Chemistry

In the second half of the 20th Century, synthesis of chemical products has experienced an exponential growth. It is therefore necessary to better assess the environmental behavior, persistence and possible toxicological effects of new synthetic organic compounds. In that sense, the present work focuses on better understanding the occurrence and fate in coastal systems of a wide variety of hydrophobic (log Kow > 3–4) personal care products (PCPs), and comparing them with well-known and persistent organic pollutants (POPs). Distributions of both POPs and PCPs have been analyzed in several environmental compartments from the highly urbanized Bay of Cadiz (SW Spain). More than 100 priority (PCBs, PAHs, pesticides from several classes) and emerging contaminants (fragrances, UV filters, insect repellents, antimicrobials, and new organophosphate flame retardants) have been measured in surface seawater and sediment samples. Additionally, caged tags (Ruditapes philippinarum) were deployed in this area during 2013 to account for the bioconcentration of selected target compounds. About 60% of the target analytes were detected in aqueous samples (up to 2.4 ng/mL). Those compounds showing the highest concentrations were synthetic fragrances such as OXONE, galaxolide and tonalide, followed by UV filters (mostly homosalate and octocrylene). Regarding priority compounds, the occurrence of PCBs was considerable, with 49% of the environmental distribution detected in sediment, over many PCPs and the rest of POPs. Other chemicals such as PCBs, pesticides, antimicrobials (i.e. triclosan) and insect repellents were only detected occasionally, and almost accounting for less than 5% of the total concentration. Levels of POPs and PCPs in surface sediments were usually between 2 and 4 orders of magnitude higher than in the dissolved phase, which can be expected due to their low solubility. Their distribution patterns revealed different sources, such as the accumulation of hydrocarbons towards the west side of the bay due to intensive road and marine traffic between cities. However, concentrations of target compounds were also related to texture and organic carbon content of sediments. Regarding biota samples, analysis of whole tissues revealed the occurrence of the same contaminants found in both aqueous and particulate phases (PCPs, POPs) and levels were significantly higher than those previously measured in non-exposed organisms, suggesting the occurrence of bioaccumulation processes.

Persistent organic pollutants in European eels (A. Anguilla) and American eels (A. rostrata): comparison between the Saint-Lawrence estuary and the Gironde estuary

M. Lauzent, LPTC-EPOC, University of Bordeaux / UMR EPOC LPTC; P. Labadie, University of Bordeaux / UMR EPOC Equipe LPTC; L. Pelletier, Université de Bordeaux / UMR EPOC LPTC; K. Lemouch, University of Bordeaux / UMR CNRS EPOC/PTC; P. Couture, INRS / Centre Eau Terre Environnement; E. Charles, Bordeaux I; H. Budzinski, University of Bordeaux / UMR EPOC Equipe LPTC

Since 1980, eels suffer from a sharp decline in their population. Many anthropogenic and natural causes are possible. However, eels by their life history characteristics are particularly vulnerable to persistent organic pollutants such as polybrominated biphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). In the present work, we present a comparative study of POP levels in American eels (Anguilla rostrata) and European eels (Anguilla anguilla). The levels of contamination of PBDEs, PCBs and OCPs were quantified in 208 muscle samples of yellow eels and 85 muscle samples of silver eels collected in the Saint-Lawrence estuary (Québec, Canada) and the Garonne-Gironde river-estuary continuum (France). In yellow eels from the Gironde estuary, significantly more contaminated by PCBs and OCPs than eels from the St. Lawrence estuary. As regards PBDE concentrations, the St. Lawrence estuary appears more impacted by PBDEs for silver eels. We also highlighted the fact that the difference in contamination between silver and yellow eels was mainly due to the significant lipid reserves of silver eels that promote the accumulation of lipophilic contaminants such as PCBs, OCPs and PBDEs.

Pyrethroid bioaccumulation in Mediterranean dolphins

Aznar-Alemany, C. Corcellas, IDAEA / Department of Environmental Chemistry; J. Gimenez, R. de Stephanis, Estación Biológica de Doñana (CSIC) / Department of Marine Biology; E. Ginestar, IDAEA-CSIC / Department of Environmental Chemistry; D. Ballester, IRiAB-CSIC / Institute for Environmental Assessment and Water Research

Pyrethroids are organic pollutants with high hydrophobicity used as insecticides. Concern exists about aquatic organisms’ exposure to their toxicity. They were believed to be converted to non-toxic metabolites in mammals, but our group has detected them in human breast milk and has proved their bioaccumulation in marine mammals and river fish. The present study investigates the occurrence of pyrethroid compounds in liver samples from striped dolphins (Stenella coeruleoalba) and common dolphins (Delphinus delphis) from southern Spain, as the first attempt to determine the occurrence and bioaccumulation and distribution of pyrethroids in marine mammal tissues from the Mediterranean Sea. Samples of dolphin tissue were collected from the Abloran Sea (south of Spain) between 2003 and 2010, including 37 liver samples from striped dolphin and different tissues—blubber, muscle, liver, brain and kidneys—from 11 common dolphins. The analytical method monitored 10 pyrethroids, including cypermethrin and deltamethrin. For the sample preparation, lyophilized sample was spiked with internal standards, extracted by sonication and underwent a clean-up with alumina and C18 SPE cartridges. Extracts were analysed by GC-NCI-MS/MS. Method recoveries for the pyrethroids ranged 53–116 % and method LODs and LOQs were 0.02-0.46 ng/g and 0.08-1.54 ng/g, respectively. Pyrethroids were detected in 87 % of the striped dolphins and 100 % of the common dolphins, with total concentrations of nd-5,210 ng/g lw and 69-2,036 ng/g lw, respectively. These levels were higher than those reported found in dolphins from Brazil (7.0-68 ng/g lw). Permethrin and tetramethrin were the main contributors to the pyrethroid profiles for all tissues. The samples of striped dolphins where used to observe that bioaccumulation of pyrethroids was unlike that of persistent organic pollutants (POPs), as pyrethroid levels were not correlated to the age of the specimens. Levels slightly increase from calves to juveniles, whereas juveniles present similar levels to adults. Metabolism of pyrethroids after achieving sexual maturity might account for this pattern. Because of the pyrethroids lipophilic behaviour, blubber was the most contaminated tissue and brain showed the lowest levels. Normalizing the data to the lipid content, the highest value was for muscle by far, suggesting a preference for that tissue.

Aquatic Nanotoxicology: from Freshwater to Seawater - overcoming the difficulties of standard toxicity tests and the implications for higher tier toxicity testing (II)

107 Effects of titanium dioxide nanoparticles on aquatic ecosystem: The first outdoor mesocosm experiment

B. Jovanovic, Ludwig Maximilians University of Munich / Chair for Fisheries Biology and Fish Diseases

During the course of the 78 days nine outdoor mesocosms, capacity 1350 L each, situated on a pontoon platform in the middle of a lake were exposed to 0 µg L⁻¹ TiO₂; 25 µg L⁻¹ TiO₂; or250 µg L⁻¹ TiO₂ nanoparticles five times a week. E171 human food additive grade TiO₂ was used environment-ally relevant concentrations arriving from wastewater treatment facility plants. Mesocosms were inoculated with sediment, phytoplankton, zooplankton, macroinvertebrates, macrophytes, and fish before exposure simulating a complete food chain. Physicochemical parameters of the water; nutrient concentrations; and the biomass of the various taxa were monitored. Concentrations of 25 µg L⁻¹ TiO₂ and250 µg L⁻¹ TiO₂ have caused reduction in the amount of the available soluble reactive phosphorus in the mesocosms by 10 % and 25 % respectively, but not in the amount of total phosphorous. The biomass of Rotifera was significantly reduced by 36 % and 48 % in the TiO₂ 25 µg L⁻¹ and TiO₂ 250 µg L⁻¹ treatments respectively when compared to the control, however the biomass of other monitored groups: Cladocera, Copepoda, phytoplankton, macrophytes, chironomids, and fish remained unaffected. In conclusion, environmentally relevant concentrations of TiO₂ nanoparticles may negatively affect certain parameters and taxaes of the freshwater aquatic ecosystem. However, these negative effects are not big enough to affect the overall function of the ecosystem as there were no cascade effects that could lead to a change in the trophic state and primary production, or in a major shift in a food web.

108 Responses of stream microbial communities to nanosilver: search for effective stress indicators

A. Tili, Eawag / Environmental Toxicology; A. Pradhan, University of Minho / Department of Biology; C. Pascoal, F. Cassis, University of Minho / Centre of Molecular and Environmental Biology (CBMA), Department of Biology; M.O. Gessner, Institute of Freshwater Ecology and Inland Fisheries

Global increases in the commercial use of nanoAg have raised concerns about the release of nanoAg into fresh waters, causing a potential risk to aquatic biota and associated ecological processes. In streams, microbial communities play a key role in organic matter turnover and energy transfer from plant-litter to higher trophic levels. However, little is known about their response to the community level to environmentally realistic concentrations of nanoAg. We investigated responses of stream microbial communities to low concentrations of nanoAg by determining i) dose- and time-response curves and ii) effective physiological indicators for oxidative stress induced by nanoAg and ionic Ag. To this end, stream microbial communities were exposed to different sources of nanoAg from natural sources (nanoAg<draft >30 nm d.m.; 4 levels <50 µg L⁻¹) for 6, 24 and 96 h. Effects of Ag⁺ (2.5 µg L⁻¹ as AgNO₃, corresponding to 5% of dissolved Ag(I) from the highest concentration of nanoAg used) were compared with those of nanoAg. Exposure to nanoAg decreased fungal species richness and reproduction in a dose- and time-dependent manner, but did not affect fungal biomass. All tested antioxidant enzymes involved in the ascorbate-glutathione cycle showed similar responses to nanoAg. Activities of superoxide dismutase (SOD), catalase (CAT),
glutathione peroxidase (GPx) and glutathione S-transferase (GST) increased with increasing nanoAg concentration and exposure time. The stress responses to Ag+ were stronger than to nanoAg at a 10 times higher concentration, but weakened with time. Higher activities of SOD and GPx indicated greater contributions of these enzymes to antioxidant defence against the stress induced by nanoAg. Overall, these results suggest that the antioxidant enzymes related to the antioxidative-glutathione cycle can play a key role in the environmental resistance mechanisms of streaminek微生物 communities against the stress induced by nanoAg at environmentally realistic concentrations. The impacts of Ag+ were more pronounced than those of nanoAg and they decreased with time. This might be related to a decline in silver bioavailability. Acknowledgements: This study was supported by the SNF through its NRP 64 program, the DAAD-FCT-2013-2014, PTDC/AAC-AMB/121650/2010 and PIDDAC-PEst-OE/BI/A/14050/2014.

109 Pollution induced community tolerance of microbial decomposers to silver nanoparticles

D. Botis, University of Minho / Centre of Molecular and Environmental Biology (CBMA), A. Tsiola, Institute of Freshwater Ecology and Inland Fisheries; C. Pascoal, F. Cassio, University of Minho / Centre of Molecular and Environmental Biology (CBMA), Department of Biology

The production and use of silver nanoparticles (AgNPs) has significantly grown over the last decade. This has increased the risk that a fraction of the NPs is transported to wastewaters and ultimately to rivers and coastal waters where NPs and released ionic silver can have toxic effects. Plant litter decomposition is an important ecosystem process promoted by both fungi and bacteria, different species of which may vary in their sensitivities to pollutants. Based on the concept of pollution-induced community tolerance (PICT), the main goal of the present study was to determine whether exposure of bacterial and fungal decomposers to low doses of nano- and ionic silver shifts their microbial community structure and thus increases community tolerance to nano and ionic silver. Microbial communities associated with leaf litter were exposed for 25 days to AgNPs (50, 100 and 200 µg L-1) and AgNO3 (20 µg L-1). Tolerance acquisition was measured in short-term bioassays to establish dose-response curves for several endpoints: fungal sporulation, bacterial production, microbial respiration and the potential activity of leucine aminopeptidase (LAP). AgNPs were stable throughout the experiment. Cluster analysis of DGGE fingerprints showed that fungal and bacterial communities were affected by long-term exposure to AgNP and AgNO3. For all pre-exposed communities, the concentration inhibiting sporulation and bacterial production by 50% was higher for nano than for ionic silver. All endpoints analysed supported the PICT concept of increased community pre-exposure to AgNPs. Bacterial communities were always more tolerant to nano and ionic silver than the control community. These results suggest that the application of PICT to aquatic microbial decomposer communities provides a powerful tool for AgNP risk assessment.

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110 The impact of silver nano-particles to planktonic microorganisms: from viruses to pico- and nano-sized protists

A. Tsiofa, Institute of Oceanography; P. Pitta, Hellenic Centre for Marine Research Crete / Institute of Oceanography; A. Callol, M. Kagiiori, I. Kalantzi, Hellenic Centre for Marine Research / Institute of Oceanography; M. Tsapakis, Institute of Oceanography

Planktonic microorganisms play a key role in global biogeochemical cycles and regulate the great majority of oceanic processes. The importance of marine microbial life raised the need for accurate enumeration of the abundance and accurate conversion to biomass of the main microbial members. Under the current scenario of generation of nano-materials flowing into the oceanic realm, the necessity to study marine microbial assemblages is particularly high. For this reason, during this project, we aimed to assess the impact of silver nano-particles (AgNPs) on the living organisms of the planktonic food web of an oligotrophic coastal area, with the use of microcosm and mesocosm experiments. To our knowledge, this is the first time that such experiments have been designed in the open ocean to include the open ocean planktonic ecosystem and study their responses to AgNPs manipulation. The interplay between viruses and pico- and nano-sized microbes (bacteria and protists) was assessed, in terms of abundance and biomass, with the use of syringe pumped and conventional air pressure flow cytometry on a daily basis. Bottles and mesocosm bags were filled with water from the bay of Gournes (Heraklion, Crete) and were finally deployed in a large concrete tank, at in situ temperature. Three incubations were not manipulated and served as controls, while the rest received a certain amount of AgNPs. Results from the microcosm experiments aided in the final decision of the AgNPs added, in order to mimic as realistically as possible their expected effect in the future; low concentrations of AgNPs showed a dissolution trend, BPEI coating (in contrast to) hindered dissolution to a greater extend than the controls, while it allowed significantly higher increase of chlorophyll. Assessing the dynamics of the key producers and recyclers of organic material in the oceans in response to elevated AgNPs is of critical importance. Abundance and biomass are crucial parameters when studying complex microbial assemblages that provide invaluable information regarding the interplay among the microbial food web components. In combination with associated measurements, such as bacterial activity assays (live/dead assay measurement) and oxidative stress response (production of reactive oxygen species), the impact of introducing AgNPs in aquatic ecosystems will be evaluated.

111 Biomarker responses and silver accumulation in fish chronically exposed to silver nanoparticles in a lake ecosystem

J.D. Martin, Trent University; V.S. Langlais, Royal Military College of Canada (Dept. of Chemistry) / Chemistry and Chemical Engineering; M.D. Rennie, University of Manitoba; C.D. Metcalfe, Trent University / Water Quality Centre

Due to the antibacterial properties of silver nanoparticles, these nanomaterials are found in an increasingly wide range of food packaging materials, textiles, household appliances, cosmetics, and medical devices. There are concerns that silver nanoparticles are entering waterways from municipal sewage systems in low (µg/L) concentrations, and their effects on aquatic ecosystems are poorly understood. The toxicity of silver nanoparticles may be due to the nanoparticle itself, the release of silver ions, or a combination of both. The goal of this research was to determine if silver nanoparticles added to a lake (i.e., Lake 222) at the Experimental Lakes Area, ON, Canada induced biomarker responses and resulted in accumulation of silver in yellow perch (Perca flavescens) and northern pike (Esox lucius) that are resident fish in the lake. Suspensions of PVP-capped silver nanoparticles were added to the lake continuously over 18 weeks (i.e., June to October, 2014) from an onshore point source. Concentrations of silver in the water column were determined by density gradient centrifugation at five sites in the lake indicated that there was gradient of concentrations across the lake, with time weighted average concentrations

112 Impact of Nanoplastic Particles on the Innate Immunity of Fathead Minnow (Pimephales promelas)

A. Greven, Chair of fish disease and fisheries biology LMU Munich; T.M. Merk, Ludwig Maximilians University of Munich / Chair for Fish Diseases and Fisheries Biology; M. Klapper, Max Planck Institute for Polymer Research / Department of Synthetic Chemistry; B. Jovanovic, Ludwig Maximilians University of Munich / Chair for Fishery Agroecology

Impact of Nanoplastic Particles on the Innate Immunity of Fathead Minnow (Pimephales promelas), was examined using neutrophil function assays. To determine the effects of PCNP and PSNP on the innate immune response in vitro application of NPs on neutrophil oxidative burst, degranulation of primary granules, and neutrophil extracellular trap (NETs) release assays were used. Application of both PSNP and PCNP (0.1 µg µl-1) each caused a significant stimulation of oxidative burst, degranulation, and NETs release of up to 30-100% increase compared to a non-treated control. This study outlines the stress response of the innate immune system of fish and indicates a potential for polystyrene and polycarbonate nanoparticles to interfere with disease resistance in fish populations.

Modelling of pesticides and biocides fate and exposure in a regulatory context (II)

113 VULPES WEB, a user-friendly web software for the evaluation of groundwater vulnerability to pesticides at territorial level

A. Di Guardo, Università degli Studi di Milano-Bicocca / Department of Earth and Environmental Sciences; A. Finizio, University Milano - Bicocca

The groundwater VULPESEmerency (VULPES) is a user-friendly, GIS-based and client-server software developed to identify vulnerable areas to pesticides at regional level making use of pesticide fate models. It is a Decision Support System developed as a web application software aimed to assist the public policy makers to investigate areas sensitive to plant protection products in order to propose limitations of use or mitigation measures. VULPES web adopts the Impact of Nanoplastic Particles on the Innate Immunity of Fathead Minnow (Pimephales promelas), which are areas characterised by the same agroenvironmental conditions (soil type, meteorology, irrigation amounts). In each UG it applies the PELMO or the PEARL model (according to user’s choice) obtaining the average concentrations
substance concentration at 1 meter depth; then VULPES creates a vulnerability map in shapefile format which classifies the outputs comparing them with the lower threshold set to the legal limit concentration in groundwater (0.1 µg/L). VULPES allows making ad-hoc modifications to several input data, such as active ingredients physical-chemical properties, crop or soil parameters in order to address user’s needs.

114 Kinetics of rapid covalent binding of aromatic amines to soil

M. Matthes, University of Osnabrueck / Institute of Environmental Research; M. Thellung, University of Osnabrueck / Physics; K. Hideg, University of Pécs / Organic and Medicinal Chemistry; H. Steinhoff, University of Osnabrueck / Physics

The spatial and temporal variability of the exposure and fate of pesticides, biocides, and other chemicals in soil and sediment is controlled by their transformation, sorption and binding processes. In biodegradation simulation studies, many of these xenobiotic chemicals exhibit a large fraction of residues, which cannot be further extracted even by harsh methods (“non-extractable residues”, NER). The paper presents a new approach of NER elucidation by paramagnetic spin probes, for investigating the kinetics of covalent binding of xenobiotic functional groups with natural soils. Luvisol soil (silt loam) and Leonardite humic acid (LHA) were incubated with the nitroxide spin label Anilino-NO (2,5,5-Triethyl-2-(3-aminothiophenyl)-pyrrolidin-1-oxy), which contains an aromatic amino functionality susceptible to interaction with soil organic matter (SOM). ESR spectra of soil samples were recorded at X-band frequency (9.43 GHz) at room temperature. A broad ESR signal was observed of Anilino-NO incubated in soil or LHA, which indicates strong restriction of the reorientational motion of the spin probe, i.e. immobilization due covalent binding of the aromatic amino group. This signal increased immediately after incubation and was used to determine the kinetics of the covalent bond formation. The maximum is approached after approximately half an hour followed by a slow decrease, which is attributed to the recombination of the unpaired electron of the NO moiety. A kinetic two-box model was used for fitting the measured values and determining the rate constants assuming pseudo-first order. The reaction of the covalent binding is very rapid in soil with a rate constant of 9.6 h⁻¹; i.e. a half-life of 0.072 hours = 4.3 minutes. The loss rate constant is two orders of magnitude smaller. Laccase-treated soil shows an increase of the amount of reactive quinones by a factor of 3, which supports the conclusion of a reaction with SOM. Both rate constants are slightly higher for the laccase-treated soil. Paramagnetic spin probing is an effective method for investigating the rapid formation of covalent binding of xenobiotic functional groups to natural soil. For chemicals containing an aromatic amino group a considerable proportion of NER can be explained by spin probes, which is important for the determination of persistence in soil and sediment required for PBET and risk assessment of pesticides, biocides and other chemicals.

115 Effect of using harsh or mild extraction methods in laboratory degradation studies with sediments: the simulated leaching to groundwater

J. Boesten, Alterra / ERA team

Assessment of leaching to groundwater is an important aspect of the risk assessment of pesticides within the EU. It is known for decades that the extractability of pesticide soil residues decreases with time. So a mild extraction method that may give about 100% recovery after 1 d contact time with the soil may give less than 10% recovery after a few months. In this study the mild extraction method used for a mild or harsh extraction method on leaching to groundwater was assessed by simulation. It was assumed that the harsh method extracted almost 100% and that the mild method extracted only half of the pesticide molecules sorbed at the non-equilibrium sorption sites. This was based on laboratory incubations with two pesticides in a sandy soil. In these studies the soil was extracted three times and the first extraction was assumed to be representative for the mild extraction method and the three extractions were assumed to be representative for the harsh extraction method. It was assumed that the degradation rate and long-term sorption parameters were derived from a hypothetical laboratory study with the topsoil (20°C, field capacity) from the FOCUS groundwater scenario Okehampton. The substance considered was assumed to have a half-life of 20 d, a desorption rate coefficient of the long-term sorption sites (kₚ) of 0.01 d⁻¹ and an fₑₑₑₑ (defined as the ratio of the non-equilibrium Freundlich sorption coefficient divided by the equilibrium sorption coefficient) of 1.0. Its equilibrium sorption coefficient corresponded with a Kₑₑₑₑ of 35 L/kg. The results of this study (calculated with a submodel of PEARL) were considered as the ‘truth’ based on the harsh extraction system. From these results the decline of the response in a mild extraction system. This decline and that of the concentration in the liquid phase were used to fit the non-equilibrium sorption parameters and the degradation rate for the mild system. This resulted in a half-life of 18 d, kₑₑₑₑ = 0.086 d⁻¹ and fₑₑₑₑ = 0.67. The leaching of this substance was calculated for the FOCUS Okehampton scenario with an annual application of 1 kg/ha in winter wheat one day before emergence. The FOCUS leaching concentration for the harsh system was 2.5 µg/L, and that for the mild system 2.1 µg/L. So the harsh extraction generated a higher leaching concentration but the effect was quite limited in this exploratory case.'n

Validation of the Shell and Scenarios Established for Pesticide Eco-risk Assessment in China through Field Monitoring

J. Zhou, Nanjing Institute of Environmental Sciences, MEP

The Pesticide Risk Assessment Exposure Simulation Shell (PRAESS) was developed by Nanjing Institute of Environmental Sciences (NIES) and Waterborne Environmental Inc. (WEI) to evaluate the potential for pesticides to occur in surface and groundwaters. It was used as the basis for the ERM pesticide risk assessment and semi-empirical evaluation of three sets of environmental fate and transport models including PRZM-EXAMS, RICEWQ-EXAMS, and PRZM-ADAM operating under the Windows environment. And currently, six exposure scenarios which can represent the environments characteristic of three typical agricultural regions in China have been incorporated into PRAESS. In order to validate the established Shell and scenario system, monitoring studies were conducted at the scenario sites during past years. Comparison of the 90th percentile of maximum daily predicted exposure concentrations (PECs) with the maximum measured concentrations of local used pesticides revealed acceptable agreement for rice-surface water scenarios, but for row crop-surface water and ground water scenarios, more filed monitoring data are required for adequate validation.

117 Conduct of a Groundwater Monitoring Study using Statistically Valid Survey Approaches to Contextualise PECGW Estimates Obtained from Regulatory Models

D. Wallace, Environmental Safety Environmental Fate; P. Sam, Syngenta Ltd / Environmental Safety Environmental Fate; P. Hendley, Phasera Ltd; A. Newcombe, ARCADIS US Inc

Current regulations governing the registration of plant protection products in the European Union (1107/2009) mandate that an assessment for the potential to reach groundwater is made, primarily using models such as PEARL and PELMO. However, monitoring programs allow the opportunity to measure real-world distributions of concentrations of contaminants in groundwaters; but the modelled estimates into context. An extensive pan-European groundwater monitoring study was initiated in 2012 to investigate and quantify the distribution of concentrations of two metabolites in very shallow groundwater beneath fields with a history of at least 3 years of herbicide use in the previous 5 years. In order to permit the extrapolation of study findings to all settings where the herbicide is used in EU, the study was designed to ensure selected sites would be spatially distributed to reflect a wide range of agronomic and climatic settings and that the site search would be focused in areas with larger amounts of maize agriculture, higher probabilities of the occurrence of shallow groundwater and in areas where the climatic conditions were favourable for higher metabolite leaching if the herbicide were applied. Pesticide groundwater modelling was conducted to examine the potential for the metabolites to leach following application to maize. The FOCUS simulation models FOCUS-PEARL (v 4.4.4) and FOCUS-PELMO (v 5.5.3) were used in the modelling study. This data is compared with the analytical data generated from groundwater samples collected from installed monitoring wells between March 2013 and July 2014. It was found that at 90% of these very vulnerable sites with a high likelihood of leaching. The modelled metabolite concentration was greater than 7.6 µg/L of metabolite A and in conclusion, careful monitoring studies designed to follow statistically valid survey sampling approaches can provide solid science based data to put model predictions into context. This study shows clearly that current regulatory modelling for polar metabolites produces PECGW estimates that greatly over-estimate real world concentrations.

118 Pesticide exposure modelling in drinking water supply catchments: opportunities, challenges and uncertainties

S. Pulman, TSGE Consulting; M. Whelan, University of leicester / Geography; I. Holman, Cranfield University / Energy and Environment

Pesticides continue to challenge Drinking Water Directive compliance in many surface water catchments used for drinking water supplies in Europe, most commonly due to diffuse-source inputs from agriculture. Catchment-scale modelling is a useful tool for assessing non-compliance risks in such catchments, for helping to identify and prioritise catchment-specific monitoring strategies and for helping to target potential catchment management interventions (e.g. restricted pesticide use on high risk soils). Although models are used for pesticide risk assessment in the EU regulatory authorisation process, these models employ standardised field-scale scenarios which do not necessarily reflect the spatial complexity of the agricultural landscape or temporal complexity of the catchment hydrological response. A small-scale catchment-scale modelling has been identified by the FOCUS Landscape and Mitigation Group as a possible higher-tier refinement to FOCUS Step 3 modelling. A simple process-based water balance model linked with a pesticide fate and transport model is described in this presentation. The model predicts daily discharge and chemical concentrations at the catchment scale for a given land use distribution and pesticide application pattern. Physical parameters were derived from the UK soil properties database. The model has been calibrated and validated successfully in five UK catchments for nine pesticide active ingredients. The challenges, uncertainties and opportunities of catchment-scale modelling during model development and model application are discussed.
Advancements in life cycle impact assessment and footprint method development (II)

119 Occupational exposures to chemicals in Life Cycle Assessment

G. Kjell, CIRAIG - École Polytechnique de Montréal / Génie Industriel; O. Jolliet, University of Michigan / Environmental Health Sciences School of Public Health; M. Margni, École Polytechnique de Montréal / Mathematical and Industrial Engineering

Life Cycle Assessment (LCA) is a comparative method that aims to assess potential environmental impacts of a product life-cycle onto several “protection areas”. It helps preventing burden shifts from one area to another while minimizing the global life impact. Human Health is one of these areas covered by LCA and notably include potential impacts on Human Health from exposures to chemicals emitted to the outdoor environment by industrial processes and activities along the product life cycle. However, indoor exposure of workers have not yet been included in the LCA framework despite its known contribution to occupational impacts. This research aims to (a) develop a novel methodology linking measured occupational concentrations to potential impacts to workers within the LCA framework, (b) expand this approach to address occupational exposure across the whole product supply chain and (c) apply these methods to a case study identify hotspots in the life-cycle and to compare the occupational exposures to population exposures due to outdoor emissions. This research shows that potential impacts due to occupational exposures to chemicals are not negligible when compared to other well-known sources of impacts on Human Health. We show that potential impact for the whole industrial sectors during one year is equivalent to the annual impacts from PM 2.5 exposures due to outdoor emissions. We also demonstrate that depending on the industrial sector the main contributor to the occupational exposures may be found along the supply chain rather than the industry itself: the relative importance of the supply chain to the potential impact on worker ranges from 10% up to more than 85%. and should therefore be included in the scope of Life Cycle Assessment. This research shows the relevance to include occupational exposures in LCA and provides an operational method allowing practitioners to include it in LCA studies. We provide direct, indirect (supply chain) generic characterization factors for occupational exposures in each industrial sectors. This enables practitioners to integrate occupational exposures to chemical in LCA. The method allocates the use of more specific data (concentrations, hours) when it is available.

120 Dynamic toxicity modelling based on the USEtox matrix framework

P. Fantke, Technical University of Denmark / Quantitative Sustainability Assessment; Q. Jolliet, University of Michigan / Environmental Health Sciences School of Public Health; C. Wannaz, The University of Michigan, Ann Arbor / SPHEHS

While it is recommended to report characterization factors for different time horizons, e.g. for infinity (steady-state) and after 100 years, the UNEPSETAC toxicity model USEtox 1.01 only allows for steady-state calculations. The present paper addresses this lack of temporal flexibility and aims to (a) develop a dynamic multimedia model based on and fully compatible with the USEtox steady-state matrix framework, (b) analyze and interpret the main parameters affecting chemical fate and exposure dynamics, and (c) evaluate whether for 3000+ USEtox 1.01 chemicals a 100 years horizon will yield considerably different results than the steady-state default assumption. To solve the dynamics of the system, we apply a classical approach based on Eigenvectors and Eigenvalues that diagonalizes the rate constants matrix. The fate factor matrix after 100 years can then be calculated as a simple matrix equation as a function of Eigenvectors Eigenvalues. Results show that for organic compounds, such as dioxin, masses in all compartments saturate (i.e. reach steady-state) within 100 days, whereas for metals, such as lead, the soil and deep ocean compartments are far from steady-state even after 100 years. The Eigenvectors and Eigenvalues also help interpreting and understanding the kinetics in each compartment. For the 3000+ organic compounds in USEtox 1.01, there is no difference in fate factors or subsequent intake fractions between 100 years and steady-state values. The only difference appears for metals fate in the deep ocean and soil, with a reduction of both fate factor in soil and related intake of agricultural produce after 100 years to between 7% and 84% of the steady-state values. We conclude that considering a time horizon for metals of 100 years raises the consistency of the LCIa results and LCI emissions from metals deposited in landfills, which can span several 1000 years.

121 Validation of the results from toxicity assessment in LCA using triangulation

S. Roos, Swerea IVF AB / Chemical Engineering; G. Peters, Chalmers tekniska högskola

The European Commission initiative for Product Environmental Footprint is based on life cycle assessment (LCA), with the USEtox consensus model as the recommended method. The connection of the scientific robustness will be crucial for the intended users to take actions based on the results. This research work aims to validate the results from toxicity assessment within the context of LCA by benchmarking USEtox with two alternative approaches in a case study. While strictly speaking there can be no experimental validation of environmental damage predicted in an LCA of a generic product, comparison of the results of three different methods can be considered a form of triangulation in LCA which can potentially provide confidence in an individual method. A textile case was chosen as the textile industry is an intense user of chemicals. Three different quantitative or semi-quantitative methods for toxicity assessments were used: the USEtox model chosen for the European PEF work; the Score System presented in the European Commission’s Reference Document on Best Available Techniques for the Textiles Industry; and the Strategy Tool presented by Ahkmah. The results show that the three methods do not give a consistent toxicity assessment of the chemicals in the case study. For USEtox the result also depends on whether the practitioner uses the default method or add more characteristics factors. It is recommended to use the default approach for chemicals with low toxicity and the quantitative approach for chemicals with high importance to the chemicals while the USEtox scores differ beyond several orders of magnitude. The Simple Score System and the Strategy Tool are very concerned with persistent pollutants and therefore the chemicals which are not readily biodegradable, receive a high score. The USEtox score on the other hand is relatively low for the persistent organic chemicals. Validation of results using triangulation can be used both to create confidence and/or help identify new challenges that were not previously perceived in the method. In this case we showed that the property of persistence is judged to have lower importance in USEtox compared to the two other methods, which is a finding that can be used to develop the fate modelling in USEtox. On the other hand, USEtox could provide additional advice compared to the two other methods, that one of the substances could be more environmentally problematic than what these semi-quantitative methods signals.

122 Approach for a Human and Eco Toxicity Indicator for Construction Products and Works (ToxScale)

P. Sahlin, BASF SE / Sustainability Strategy; F. Kalberlah, FoBiG GmbH; Q. Jolliet, BASF SE; B. Grahl, FA, Integrall; E. Schmincke, PE-International

Currently, potential toxicological and eco-toxicological impacts of construction products and works are insufficiently characterised. There are no meaningful indicator(s) for a comparative ranking to assist declaration, selection and substitution decisions. At least at present, characterisation models like USEtox have serious limitations to adequately describe potential toxicological and eco-toxicological impacts for a complex life cycle of construction products with heterogeneous exposure conditions from cradle to grave. One reason is the very limited availability of qualified data. However, substance information generated within REACH registration could provide a wealth of useful data to describe health and eco-toxicological impact. Because of the different philosophies of REACH and LCA, characterisation models may be difficult to directly map to the use of REACH data within LCIA. Therefore, an indicator is proposed, which is applicable in Environmental Product Declaration (EPD) providing additional human health and eco information to LCIA according to ISO 14025. This indicator, “ToxScale”, uses REACH information for toxicological and eco-toxicological characterisation of construction products. This paper provides an outline of the ToxScale approach. At this stage, discussion is restricted to human health impact, but may be extended to eco-toxicity. ToxScale is characterised with: 1) a hazard score based on “derived no effect level” (DNEL) 2) an exposure modifier related to the REACH “risk characterisation factor” (RCR), 3) REACH specific descriptors for environmental release categories (ERCs) and process categories (PROCs) to quantify emissions with potential impacts for all stages of a substances’ life cycle, 4) an aggregation procedure aggregating along different life cycle stages, and 5) a supplemental procedure to address substances, exposure scenarios, life cycle stages and inherent toxicity, which are not adequately addressed under REACH, but may contribute to the potential impact of a construction product. ToxScale integrates REACH thinking and LCIA thinking as well as “risk” and “hazard” thinking to build an indicator, which permits construction products’ ranking based on potential health and eco-toxicological impacts as a supplement to LCIA.

123 Strategies to deal with information of different reliability exemplified by the use of QSARs to fill the algae data gaps in LCIA of plastic additives

K. Jadhalkar, Ph.D; Department of Studies in Environmental Science; T.V. Rydberg, M. Rahmberg, IVL Swedish Environmental Research Institute; H. Anderson, IVL Swedish Environmental Research Institute Ltd / Department of Chemical and Biological Engineering; U. Sahlin, Linnaeus University, School of Natural Sciences

Data gaps are problematic when screening for dangerous substances or in impact assessment where several chemicals are considered for evaluation. Non-testing information can be replaced by non-testing information such as Quantitative Structure Activity Relationships (QSARs), but even though this latter information comes with lower reliability, this is seldom taken into account in the forthcoming assessments. The difficulty to meet standards for best information calls for strategies to handle data gaps which take varying reliability in information into account. Using safety factors when reliability is low can be problematic since this result in more conservative evaluations of substances for which information is of low reliability and an unknown level of risk aversion in the assessment. An alternative is to reflect lower reliability using probability distributions representing the expected error in the information and propagate this uncertainty in the

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forthcoming assessments using Monte Carlo analysis. It is even possible to let the technology interactions and associated environmental impact of such systems has been applied in this context. LCA plays a core role in the criteria development process for assessing environmental impacts of typical products on the market, identifying life cycle hotspots and evaluating improvement options. Review of the material produced in the literature and development of ad-hoc studies to integrate such information can be functional for the definition of key environmental areas on which to set criteria and for addressing further investigation efforts to find best practices of production and consumption. A complementary product-oriented and technology-related perspective is necessary to gather preliminary information for the development of ad-hoc environmental elements that could be not entirely integrated in LCA (e.g. legislative constraints, market and quality of products, inherent safety, indoor air quality, noise emissions) and to identify options which could be used to formulate relevant criteria. In this process, interaction with stakeholders is essential for gathering information and validating experts, providing a role in the definition of feasible and fair criteria. The outlined approach has been applied to a series of product groups. As illustration, main findings related to the development of labelling criteria for Absorbent Hygiene Products (AHP), which includes products as baby diapers, feminine care pads, tampons and nurse pads, have been reported. The criteria development process involves the analysis of complex systems where LCA plays a core role, although the definition of a coherent set of criteria needs also multi-disciplinary analyses and continuous discussion with experts. LCA can be a powerful instrument for informing policy makers but there are inherent limitations which require critical analysis and certain flexibility for finding a balance between what could be good to do in theory and what can be done in practice.

126 Consequences of changing modelling choices on complex data systems G. Wernet, ecovent Centre; E. Moreno Ruiz, ecovent In LCA, the choice of goal and scope determines which modelling choices are appropriate for a study. An LCA study also needs both detailed LCI data on the foreground processes and reliable background information. Attributional impact assessment methods are recommended, although several have emerged in the last years. However, consequential databases have been scarce, and most LCA databases are published following only one specific set of modelling choices, which limits their applicability to studies where the choices are acceptable within the goal and scope definition. This presentation describes a system that allows multiple sets of modelling choices on the same underlying process data. The ecovent database version 3.1 is published in 3 system models: Allocation, Cut-Off, Allocation, Point of Substitution (APOS), and Consequential. By comparing the LCIA results of the same LCI database with changing modelling assumptions, the consequences of the modelling choices can be directly observed over a complex system of over 10000 LCI datasets. A comparison of the results over the whole database reveals that in more than 60% of cases, the choice of allocation method has only minor consequences when comparing Cut-off and APOS. The 2 models can thus form the boundaries for sensitivity analyses of allocation method choices. In comparison, the attributional and consequential models show strong differences in results in many cases. An analysis reveals that 5 common reasons can contribute to differences: the marginal suppliers, activities involving use of or production of many by-products, joint production and by-products that substitute very harmful or very clean production routes. Here the consequential model offers a further perspective and is the only model suitable for studies where the goal and scope demand a consequential viewpoint. The model is thus a significant improvement for consequential studies. Applying different system models to complex systems offers new analysis options in LCA studies. Studies can profit greatly from 2 different allocation perspectives and a consequential perspective as well. For attributional studies, the choice of allocation approach can be relatively unimportant, whereas the results of attributional and consequential studies often differ a lot. The availability of 2 allocation models and a consequential model, all based on the same underlying data, allows for a better and more thorough understanding of LCA results of complex systems.

127 Needs and Challenges in Life Cycle Assessment of Integrated Energy System X. Zhang, Laboratory for Energy Systems Analysis; C. Bauer, Group Environment, Health & Safety; C. Mutel, Paul Scherrer Institute / Laboratory for Energy Systems Analysis Life Cycle Assessment (LCA) is applied in most applications to assess the environmental impact of an individual product or technology. However in reality, planners and decision makers are often challenged with complex energy system with multiple technologies for energy generation, demand management, energy storage, further integration of fluctuating and renewable energy sources, and operation conditions. Decisions have to be made not only on the level of individual technologies, but with consideration of techno-economic performance and environmental impact of the entire system as a whole. LCA is a crucial tool to assess the technology interactions and associated environmental impact of such systems, and can generate meaningful and quantitative results that help planners to make more informed decisions. With the definition of integrated energy system, the assessment framework for such system has been developed: procedures for performing integrated energy system assessment have been identified, with focus on addressing the challenges and experience of doing LCA for individual technology and for the entire energy system. Differences in comparison with
conventional LCA applications are discussed in detail. Demonstrations on practical applications are presented using energy technologies explored within the project scope of SCCER-HaE (Swiss Competence Center for Energy Research - Heat and Electricity Storage[1]).

128 Integrating LCA and Scenario Modelling of the Energy System for Sustainable Policy-Making
K. Volkart, Paul Scherrer Institut / Laboratory for Energy Systems Analysis
Today, life-cycle assessment (LCA) is frequently used to support policy-making in strategic decision problems. LCA has been developed for the environmental assessment, i.e. it includes a variety of environmental decision criteria. It typically only evaluates measures or processes, i.e. lacks the system perspective. Therefore, LCA should be extended or combined with other tools to more comprehensively support policy-makers in their decisions related to complex systems such as the energy system. The energy system can be entailed a complex system considering features such as large-scale integration of new technologies, substitution between energy carriers and electricity grid stability. Additionally, this techno-economic system is embedded in the political and social context (e.g. climate change mitigation, air pollution prevention and energy access). In many countries energy policy-making is at the crossroads: new energy strategies considering the abovementioned societal boundary conditions have to be developed to guide the energy system into a (more) sustainable future. To visualize possible futures, various scenarios have been described and quantified using so-called energy-economic system models. Such models include the entire system and its techno-economic characteristics but they do not address other detailed criteria such as those considered in LCA. Therefore, the goal of this work is the development of a methodology for the integration of LCA and scenario modelling. Such an approach combines the system-wide and economic perspective with the detailed technology assessment of LCA. In doing so, LCA information is used to overcome the lack of detailed impacts on the processes and policy decisions represented in the scenarios, and thereby allows for more robust decision-making. The proposed methodology is applied and tested in a full-scale energy system economic system model. The complete life-cycle burdens of the processes in the energy system are taken into account, i.e. direct emissions, impacts of the construction and dismantling of the infrastructures, and impacts of the materials and fuels used. Also, the underlying heat, electricity and freight transport technology mixes are modelled scenario-dependently. So overall, this work is a novel approach for the use of LCA in the analysis of complex systems that can provide to more comprehensive and more complete information for energy policy-making.

129 Top down vs Bottom up to model complex systems: rational and irrational behaviours
T. NAVARRETE GUTIERREZ, CRTE CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE; S. Rege, Luxembourg Institute of Science and Technology (LIST) / Resource Centre for Environmental Technologies CRTE; A. Marvuglia, Luxembourg Institute of Science and Technology (LIST) / Resource Centre for Environmental Technologies CRTE; M. Stefanova, ENEA / LCA and Ecodesign Laboratory; C. Tripepi, ENEA
Top-down vs Bottom-up model (ABM). Each model evaluates the environmental consequences of augmenting maize production by 80000 tons (termed as shock) to generate one additional (CNS). Morever, those behaviours can be impaired or lost as a result of exposure to fish, its association with biochemical alterations and related impairments of fish. At least for copper, behavioral responses of fish generally occur within a range of copper concentrations comparable to those associated with reproductive and brain behaviour patterns (i.e. locating food, avoiding predators) are triggered by the analysed system, measure their effects and express the results in terms of sustainability indicators. Finally, the analysis should be able to integrate empirical facts (i.e.: emissions, re source use, market shifts, etc) with normative positions (i.e.: reduction targets, legal thresholds, value choices). Keeping the life cycle approach and methods as reference, an integration framework for sustainability has been developed, namely Life Cycle Sustainability Analysis (LCSA), which is based on an inconsistent logical combination of technical, economic and social norms, and thus is in line with the requirements described above. However, while it has been set up and defined at ontology and epistemology level, it is still not operational [1, 2, 3]. The framework recognises the key role of the assessment in the goal and scope definition phase, where the sustainability questions are defined and the mechanisms of the system to be modelled are identified. This paper presents an approach to structure the Goal and Scope phase of the LCSA framework [4], considering the application to the development of a new technology system for the production of high-purity hydrogen from biomass gasification, to be used in fuel cells for automotive transport. The technology is currently being developed in the UNIHY project, funded by the Fuel Cells and Hydrogen Joint Undertaking (FCH JU) initiative within the 7th Framework Programme of the European Community.

Behavior Revised: Examining Behavioral Effects of Contaminants and Other Stressors in Aquatic Animals
A Review of Copper Effects on Fish Behavior
Concerns have been raised regarding the potential adverse effects of copper on fish behavior at concentrations lower than U.S. Environmental Protection Agency (USEPA) water quality criteria. To evaluate these concerns, USEPA performed a literature review of copper effects on the behavior of both freshwater and marine fish. Information on the behavior of 58 freshwater and 16 marine fish species was found, with a combined total of 180 LOEC’s and 53 NOEC’s. Laboratory studies with copper alone, as part of mixtures, and field behavioral studies were all reviewed. Of the 129 LOEC values for laboratory studies with copper only exposures in freshwater, 125 (97%) were higher than the USEPA hardness adjusted chronic copper criterion. All 24 laboratory LOEC’s for marine fish exposed to copper alone were higher than the USEPA marine chronic criterion. Avoidance behavior is the most commonly studied endpoint, and is the only endpoint with LOEC’s lower than the freshwater chronic copper criterion. Fish avoid copper at low concentrations, but are attracted to copper at higher concentrations. Other behavioral endpoints with available copper effects data include voluntary and involuntary movements, swimming, feeding, social, reproductive and respiratory behaviors. At least for copper, behavioral responses of fish generally occur within a range of copper concentrations comparable to those associated with reproductive and growth effects of copper on fish, meaning the existing USEPA hardness dependent copper criterion is not acceptable protective from adverse behavioral effects.

132 Behavioural effects of inorganic mercury on white seabream (Diplodus sargus) together with accumulation levels and biochemical changes in eyes and brain
P. Pereira, Department of Biology and CESAM; J. Raimundo, IPMA / DIVOA; F. Feio, Biology Department of Aveiro University / CESAM Department of Biology; M. Barata, Portuguese Institute for the Sea and Atmosphere; O. Araújo; V. Cardoso, F. Pinto-Ribeiro, University of Minho / Life and Health Sciences Research Institute ICVS School of Health Sciences; P. Pousão-Ferreira, Portuguese Institute for the Sea and Atmosphere; M. Santos, Aveiro University and CESAM; J. Canario, Instituto Superior Técnico / Centro de Quimica Estrutural; A. Almeida, Life and Health Sciences Research Institute ICVS School of Health Sciences; University of Minho / Life and Health Sciences Research Institute ICVS; M. Pacheco, University of Aveiro / Dept of Biology
Mercury (Hg) compounds have triggered major environmental and human health concerns. Still, there is a knowledge gap on Hg accumulation in brain and eyes of fish, its association with biochemical alterations and related impairments of behaviour. Fish behavioral patterns (i.e., locating food, avoiding predators) are mediated by eyes with an appropriate integration of the central nervous system (CNS). Moreover, those behaviors can be impaired or lost as a result of exposure to Hg. Additionally, no data exist on the ability of those organs to eliminate Hg in a...
post-exposure period, as well as on recovery of biochemical endpoints and, consequently, on the reversibility of behavioural syndromes. Current study was designed to mitigate such lack of information by combining the swimming performance of the white seabream (*Diplodus sargus*) together with Hg accumulation and biochemical endpoints (oxidative stress biomarkers and acetylcholinesterase – AchE) in eyes and brain. Fish were exposed to realistic levels of inorganic Hg in water (2 µg L⁻¹) during 7 (E7) and 14 days (E14). After that, fish were allowed to recover for 28 days (PE28). The Fulton condition and hepatosomatic indexes did not vary significantly between control and exposed fish along the experiment. At E7, exposed fish exhibited a significant decrease of the first swimming distance, as well as a lower time for refuge. In parallel, Hg levels were significantly higher in brain and eyes, while both activities of AchE and GPx in brain and eyes were significantly decreased. At PE28, previously exposed fish still swam a shorter distance in the first run, exhibited a lower resistance against the water flow (measured as time for the immobility) and lower latency immunity. Accordingly, Hg levels in eyes and brain did not decreased during the recovery period and GSH levels were augmented in brain. Realistic levels of waterborne inorganic Hg can alter fish swimming performance without a rapid reversibility. Such behavioural impairments are in agreement with Hg accumulation in fish brain and eyes, as well as biochemical alterations in brain, which may have repercussions in organism’s fitness and survival.

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**Seasonal troubled waters: The impact of trenbolone on sexual selection in a freshwater fish**

P. Tomkins, Monash University / Biological Sciences; M. Saaristo, Monash University / School of Biological Sciences; M. Allinson, The University of Melbourne / School of Chemistry; B.B. Wong, Monash University / School of Biological Sciences.

Sexual selection and female mate choice are particularly sensitive to human-induced environmental change. It has been well established that changes to the visual and acoustic environment can affect the ability of females to choose mates, however the consequences of altering the chemical environment are relatively unknown. Endocrine disrupting chemicals (EDCs) are one group of chemical contaminants with the ability to disrupt sexual selection. A recent surge in interest towards behavioural studies has revealed that EDCs can impact a wide range of behaviours, but their effect on sexual selection and female mate choice is poorly understood. Further, the majority of behavioural ecotoxicological studies have focused on only one group of EDCs (i.e. those with estrogenic properties), while comparatively little is known about the behavioural impact of androgenic EDCs. One androgenic EDC with the potential to affect sexual selection and female mate choice is trenbolone. Trenbolone is a particularly potent androsteroid compound used to promote growth rates in beef cattle around the world, and has been detected in waterways associated with cattle feedlots. It is known to cause severe morphological and physiological abnormalities, but its impact on sexual selection has yet to be investigated. To address this gap in the literature, we investigated the impact of an environmentally relevant concentration of trenbolone (40g/L – 21 day exposure) on female mate choice in the guppy (*Poecilia reticulata*). When given a choice between trenbolone-exposed and unexposed males, unexposed females were found to prefer unexposed males. However, trenbolone-exposed females showed no preference for either male, suggesting they had lost the ability to discern between males of differing quality. Considering female mate choice plays a pivotal role in maintaining the genetic fitness of a population, their results could have far reaching evolutionary consequences. This is one of the few studies to show that an androgenic EDC can influence female reproductive behavior, and the first to show that androgenic EDC exposure can affect sexual selection and female mate choice.

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**Antidepressants alter aquatic predator-prey interactions**

M.L. Hedgespeth, A. Nilsson, O. Berglund, Lund University / Dept of Biology

Because many antidepressant pharmaceuticals are detected at levels too low to exert direct lethality in the environment, it is necessary to explore those sublethal endpoints, such as changes in organism behavior, that can give rise to effects at the population/community level, providing a vital link to interpreting the effects of environmental contaminants on population and community dynamics. Such data can be used along with the application of simple ecological models for predicting ecological effects at the population/community levels in aquatic systems.

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**Biocorrelation of phenanthrene and metabolites in bile and behavioral changes in the tropical estuarine guppy *Poecilia vivipara***

P.S. Carvalho, A.G. Torreiro-Melo, UPPE Universidade Federal de Pernambuco / Department of Zoology; J. Silva, Sao Paulo State University - UNESP / Eng Ambiental; A. Bianchini, Universidade Federal do Rio Grande - FURG / Instituto de Ciências Biológicas; E.Z. Lamardo, Universidade Federal de Pernambuco

Quantification of polycyclic aromatic hydrocarbon (PAH) metabolites in fish bile is widely used to evaluate levels of internal PAH contamination in fish, whereas behavioral effects are deemed important to address potential risks to fish populations. The estuarine guppy *Poecilia vivipara* was exposed for 96 h to waterborne phenanthrene at concentrations of 10, 50, 200 and 500 µg L⁻¹. Physiological and behavioral endpoints were assessed in bile samples, linking insecticide exposure with 260/380 nm (excitation/emission) wavelengths. Phenanthrene and metabolites increased in the bile of exposed fish in a dose-dependent pattern, and log bile bioconcentration factors ranged from 4.3 to 3.9 at 10 and 50 µg L⁻¹ phenanthrene, respectively, values that are similar to predicted bioconcentration factors based on phenanthrene Kₐ. Swimming resistance index was reduced to 81% of control values at 500 µg L⁻¹. Activation of swimming speed was non-reversible, with significant speed increase relative to control fish in treatments 50 and 200 µg L⁻¹ phenanthrene, respectively, followed by a speed decrease in fish exposed to 500 µg L⁻¹. The search volume model of fish foraging behavior is based on the idea that the probability of prey detection and capture increases as a predator fish swims faster and therefore for longer distances searching for prey, exploring a larger volume of the habitat for prey. However, in this study the ability of *P. vivipara* to capture prey organisms was diminished in the 200 µg L⁻¹ phenanthrene treatment where swimming speed increased. Swimming trajectories of fish exposed to 50, 200 and 500 µg L⁻¹ was altered by the development of a repetitive circular swimming behavior, in contrast to the controls that explored the entire experimental area. Interestingly, the change in swimming behavior appeared to be induced by the increase in capture rates at 200 µg L⁻¹ phenanthrene. These results indicate that a change in the pattern of habitat exploration may be more important than changes in swimming speeds to predict consequences to prey detection and capture. The results obtained provide dose-dependent relationships between doses of phenanthrene and metabolites in bile of *P. vivipara* and behavioral effects of greater relevance to the predators, with the potential to affect prey organisms in an ecologically relevant behavioral effects in the species, which can be of significant utility in studies of environmental monitoring of oil pollution in tropical regions.

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**Oil sands process-affected water and fish sensory responses**

C.B. Trenney, M. Reichert, T. Gabusch, University of Alberta / Biological Sciences; J.B. Blum, University of Alberta / Department of Biological Sciences; B. Sutherland, University of Alberta / Physics; M. Gamal El-Din, University of Alberta / Department of Civil and Environmental Engineering

Oil sands process-affected water (OSPW) is a complex mixture containing metals, salts, naphthenic acids (NAs) and other compounds. The sensory neurons that fish use to guide so many critically important behaviors are known to be sensitive to metals and salts, but their sensitivity to NAs remains unknown. What we were curious about is whether OSPW and OSPW that had been ozonated (a putative detoxification treatment), constituted an ‘odorant’ that (1) evoked sensory responses, (2) modified sensory responses to other odorants (i.e. caused olfactory impairment), and (3) evoked behavioral avoidance. Our data suggest that sensory neurons can detect OSPW and ozonated OSPW, that some dilutions of OSPW can impair sensory responses, and that fish may elect to move away from point sources of OSPW. Our findings have implications for both the sensing of OSPW by aquatic vertebrates and the possible impact of OSPW on fish in a natural setting.

**Evolutionary, multigenerational and epigenetic effects of pollutants: scientific support to long-term ERA**

Population genome tools for quantifying evolutionary outcomes and their potential for risk assessment

Indianola University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; C. Jackson, N. Keith, Indiana University Bloomington; S. Glaholt, Indiana University; J. Colbourne, University of Birmingham; J.R. Shaw, Indiana University / The School of Public and
Environmental Affairs and The Center for Genomics and Bioinformatics

Differences in inter-individual response are described by variation in environments and genomes that combine to give rise to phenotypic variation observed in populations. Population genomics provides tools for understanding these features that provide a link to phenotype and could be useful to risk assessors. In this talk we draw from recent population genomic studies to explore how toxicant exposure contributes to genome variability, influences the fate of genome variation of populations, and how factors and the link time scales determine the CNV and phenotypes within populations. These studies contribute to and make use of a maturing genomic tool kit for Daphnia pulex, that includes array comparative genic hybridization (aCGH) and full-genome re-sequencing of over 50 individuals. Using this model of structural variation we report exposure-induced alterations in the genotype and distribution of genic environment in natural populations. We reveal a method for measuring the contribution of this environment induced genome variation on phenotype, and through spatial and temporal studies determine that CNV play a major role in establishing the environmental stress-response of a population. Finally, we discuss the importance of understanding genome variation and the evolutionary forces that shape it, and demonstrate how population genomic tools can be used to quantify the evolutionary forces acting on populations so that they could potentially be applied to risk assessment.

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Chronic radiation exposure in plant populations: from molecular to population effects

S.A. Geraskin, Russian Institute of Radiology and Agroecology / Radioecology

The consequences of long-term chronic exposure to low pollutant concentrations is neither well understood nor adequately included in risk assessments. To understand effects of real-world contaminant exposure properly we must pay attention to what is actually going on in the field. However, for many wildlife groups and endpoints, there is little evidence that link time scales determine the CNV and biological effects in natural settings. To fill the gaps, results of field studies carried out on different plant species (winter rye and wheat, spring barley, oats, Scots pine, wild vetch, crested hairgrass) in various radioecological situations (nuclear weapon testing, the Chernobyl accident, uranium and radium processing) to investigate effects of long-term chronic exposure to radionuclides are discussed. In spite of high heterogeneity in response, we have detected several general patterns. Plant populations growing in areas with relatively low levels of pollution are characterized by the increased level of both cytogenetic alterations and genetic diversity. Accumulation of cellular alterations may afterward influence biological parameters important for populations such as health and reproduction. Presented data provide evidence that in plant populations heavily contaminated territories cytogenetic damage were accompanied by decrease in reproductive ability. In less contaminated sites, because of the scarcity of data available, it is impossible to establish exactly the relationship between cytogenetic effects and reproductive ability. Contamination of the plants environment activates genetic mechanisms, changing a population’s resistance to exposure. However, there are ecological situations in which enhanced resistance has not evolved or has not persisted. Consequently, there are good theoretical and practical reasons for more attention being paid to the mechanisms by which populations become more resistant and to those situations where adaptation appears not to be taking place. Since adaptation plays an important role in response of populations on radiation exposure, this process needs to be incorporated into management programmes. To this very day, the effects of chronic exposure on living organisms and populations remain poorly explored, and represent a much needed field of research.

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Long-term effects of metal pollution in freshwaters: can genetic diversity of Chironomid populations be an indicator of environmental quality?

L.A. Pedrosa, CESAM / CESAM; Departamento de Biotecnologia; A.L. Machado, University of Aveiro / CESAM Department of Biology; B. Cocchiaro, Senckenberg Research Institute; D. Campos, Department of Biology and CESAM; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; C. Nowak; J.L. Pestana, CESAM & University of Aveiro / Department of Biology and CESAM.

Anthropogenic pollutants are well-known selective pressures with the potential to cause detrimental consequences to the long-term viability of freshwater biota. Environmental risk assessments are usually based on standard laboratory tests which evaluate potential harmful effects of pollutants on a limited number of model species under controlled conditions. Although being critical for ecotoxicological evaluation of direct effects, these approaches do not always reflect diversity and thus limit the assessment of natural ecosystems’ health. Moreover ecological indicators, based on assemblages of species are useful for monitoring the quality of freshwaters but fail to address important questions related to the dynamics and future ecological condition of aquatic ecosystems. For example, do anthropogenic stressors alter the genetic diversity of natural populations? If so, is there any associated fitness cost that affects the ability to cope with environmental changes? Are some populations adapted to anthropogenic stressors? During the last decades there has been increased interest in the application of population genetic markers in an ecotoxicological context. It is believed that depending on the duration and intensity of pollution, reductions of genetic diversity may occur in natural populations inhabiting polluted areas as a consequence of changes in population structure. Because the ability of natural populations to adapt to pollution is directly related to the amount of genetic diversity, contaminant-driven genetic erosion may counteract potential genetic adaptation and threaten the long-term viability of populations under environmental changing conditions. Therefore, genetic diversity as an indicator of environmental condition may add value to ecological risk assessment. Although numerous studies have evaluated the effects of contaminant exposure on levels of genetic diversity in aquatic organisms, there are still limited evidences on how genetic diversity measures correlate with other ecosystem level indicators such as biotic indexes. In order to evaluate the applicability of genetic diversity measurements as ecological indicators, we investigated how historical metal pollution affected genetic diversity of natural populations of Chironomus plumosus. It was found that, if there was evidence of increased tolerance to metals and if levels of genetic variation were correlated with macroweberior structural and functional diversity.

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Chronic depleted uranium exposure effects DNA methylation in zebrafish K. Remballe, Institut de Radioprotection et de Sûreté Nucléaire - PRPENVERSISLECO; S. Pereira, IRSNPRP-ENV / SERISLECO; J. Ravenat, CEA Grenoble; V. Camilleri, Laboratoire de Radiologie et d’Ecotoxicologie, Institut de Radioprotection et de Sûreté Nucléaire; I. Cavaille, IRSN; J. Bourdineau, Université Bordeaux; C. Adam-Guillermin, IRSN

Among environmental pollutants, uranium (U) is an actinide naturally present in the environment. Although being critical for risk assessment, the (eco)toxicity of this metal has been well studied, but, despite the large amount of knowledge accumulated until there, some are lacking, and particularly how U impacts gene expression and the possible risk in a transgenerational context. Many factors, as the appearance of DNA mutations play a role in the aetiology of several biological dysfunctions, but these factors also include the chemical modifications of the DNA (without changes in the sequence) and epigenetic modifications denominated as ‘epimutations’. DNA methylation is the epigenetic mechanism which has been the most studied and is known to be highly related to gene expression. In this context, we decided to study DNA methylation patterns induced by a chronic exposure to environmental concentrations of depleted uranium to evaluate the possible link with the effects already observed on gene expression in freshwater model, Danio rerio. In order to better characterize environmental problematic of uranium contamination, male and female zebrafish were exposed separately to two environmentally relevant waterborne concentrations of depleted uranium (DU) : 2 and 20 µg L\(^{-1}\). During 24 days. DNA methylation was assessed using MS-AFLP, method based on the difference in sensitivity to cytosine methylation between two endonucleases which share the same cleavage site (MspI, HpaII). Global methylation levels measured through the experiment revealed that DU induced gender-specific hypomethylation patterns which were mostly found in exposed males, with a strong dose-dependent impact, and particularly, the highest decreases were observed for those exposed to 20 µg L\(^{-1}\). Impacts on methylation patterns were not related to the intra-tussular concentration of U. These results highlight for the first time that the long-term relevant concentration of DU can induce modifications of DNA methylation in the freshwater model Danio rerio. The hypomethylation of enzymes recognition sites occurred mostly in exposed males in a dose-dependent manner and revealed a gender-specific response to DU contamination. As DNA methylation is impacted in gonads, these patterns of modifications could be transmitted to the offspring, impacting its development. Furthermore, to determine if it impacts sequences involved in gene expression, we plan to study DNA methylation by McDIP-Chip analysis.

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Multigenerational effects of contaminants in D. magna. Effects of offspring quality

C. Barata, CSIC / Environmental Chemistry; B. Campos, IDAEA-CSIC / Environmental Toxicology; R. Jordão, Biology; M.F. Lemos, Instituto Politécnico de Leiria / Dept Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM

Most ecotoxicological test guidelines are only considering effects within one generation, thus potential effects across the successive generations are usually under evaluated. The aim of the present study is to set up a protocol to perform a multigenerational ring test using the existing guidelines for the Daphnia magna reproduction test. One of the most important factors to be considered is when the second generation starts. It is well known in ecology that the size and quality of offspring varied across the first clutches in D. magna and that certain chemicals affect the offspring's quality at different stages. In this work, three contaminants nonylphenol, pipertonyl butoxide (PBO) and tributyltin (TBT).In all three chemicals chronic toxic effects on reproduction were more pronounced in the second generation. Life-history analyses showed that the studied chemicals affected the quantity and quality of the offspring produced by exposed females and a result when those offspring was further exposed showed greater sensitivity than the parental generation. These results are in line with those obtained in multigenerational studies in mammalian tests that evidenced that in most cases effects on the second generation can be predicted evaluating the quality of the offspring produced. Acknowledgement-This project was supported by the Spanish MEC grant CTM2011-30471-C02-01. The Portuguese Foundation for Science and
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**Metabolomics and Pollution Sourcing: Molecular Markers in the Field**

142 Metabolomics reveals a perturbation to kairomone chemical messengers in ZnO nanoparticle exposed Daphnia magna

N.S. Taylor, Wilfrid Laurier University / Biology; J. Sostare, University of Birmingham; F. Fabrega, Universitat Rovira i Virgili / Chemical engineering department; U. S. SIDEW, University of Birmingham / geography earth and environmental sciences; J. R. Lead, University of South Carolina / Center for Environmental NanoScience and Risk; C.R. Tyler, Biosciences College of Life and Environmental Sciences; M. R. Viant, University of Birmingham / School of Biosciences

Zinc oxide nanoparticles (ZnO NPs) are one of the most widely used nanomaterials, applied in consumer products from cosmetics to electronics. Nevertheless, our understanding of their aquatic toxicity is limited, although it is speculated to arise from dissolution of ZnO from the NPs. Metabolomics, the study of the small molecule composition of biological samples, is a proven approach for discovering metabolic responses to toxicants. We exploited the non-targeted capabilities of metabolomics to investigate the molecular toxicity of waterborne ZnO NPs to *Daphnia magna*. Mass spectrometry based metabolomics showed a dose-dependent metabolic response to the NPs, orthogonal to the effects of bulk ZnO and ZnO$_{no}$ dissolution. Detailed investigations, including MS/MS for metabolite identification, revealed that families of aliphatic sulfates and sulfamates were significantly decreased (up to 10-fold) following ZnO NP exposure. Some of these compounds have previously been shown to act as kairomones; chemical messengers emitted by the *Daphnia* that benefit another species without benefitting the emitter. Kairomones released by the daphnids are sensed by some algal species, which subsequently alter their morphology in direct response to this predation threat. Using the unicellular alga *Desmodesmus subsipicata*, we have replicated this morphological response to the kairomones of healthy *D. magna*, and more importantly have shown a modulation of an algal morphological response following exposure of *D. magna* to ZnO NPs. In addition, we have used several imaging modalities to reveal the uptake of ZnO NPs into the *Daphnia* gut and associated cellular perturbations that underpin this toxicity. Collectively these discoveries represent a novel NP-biota interaction with currently uncharacterised ecological consequences. Here we present the draft mapping of this nanotoxicological effect onto an adverse outcome pathway framework that spans two trophic levels.

143 1H NMR-based metabolic profiling reveals tissue-specific responses to mercury toxicity in wild fish Liza aurata

J. Campelo, University of Messina / Biological and Environmental Sciences; P. Pereira, Department of Biology and CESAM; M. Maisano, University of Messina; J. Raimundo, IPMA / DIOVA; A. Mauceri, University of Messina / Biological and Environmental Sciences; M. Pacheco, University of Aveiro / Dept of Biology Among the heavy metals, mercury (Hg) is a hazardous pollutant due to its persistence, bioaccumulation, toxicity to organisms, and subsequent ecological risk. In order to elucidate the toxicological responses induced by Hg in fish, an environmental metabolomics approach was applied in golden grey mullet *Liza aurata* collected from a Hg impacted area of the Ria of Aveiro, Portugal (Laranjo, LAR), and a reference site not known to be polluted by Hg (São Jacinto, SJ). Physico-chemical parameters were measured at both sites. Total Hg (tHg) and methylmercury (MeHg) were measured in water and sediment samples, and confirmed that LAR is a highly Hg contaminated area. In liver and gills of fish from LAR, higher levels of Hg and MeHg were found in respect to SJ, evidencing an Hg exposure. Also, MeHg and Hg accumulation were respectively 10-fold and 17-fold higher in liver than gills. 1H NMR spectroscopy and chemometric analysis were applied on fish liver and gills. The metabolic profile of liver were dominated by taurine and glycophosphocholine, while gills by taurine and lactate, and several other changes in classes of compounds were detected. PCA score plots described the majority of variance between spectra (i.e. 78% in liver and 71% in gills) and clustered samples with similar metabolite profiles, clearly separating the two fish groups. The metabolic profile of livers revealed increased phosphocholine and glycophosphocholine, suggesting perturbation in osmoregulation; increase in hepatic glucose indicating glycogenolysis; and increase in amino acids resulting in ammonia production. In gills the elevation of glutathione and glutamate involved in oxidative stress defence. In gills the elevated choline level may result in alteration of lipid metabolism; increase in creatine may support long-term energy demand; and decrease in dopamine may be a result of dopaminergic tone. 144 Characterization of fluvial biofilm exposed to multi-stress conditions using a metabolomics approach

A. Sessi, Water Quality; D. A. Muñoz, Catalán Institute for water research (ICRA) / Water Quality; N. Corcoll, Department of Biological and Environmental Science; B. Huerta Buitrago, Catalan Institute for Water Research (ICRA) / Department of Water Quality; S. Rodríguez-Mozaz, Institute for Water Research (ICRA) / Water Quality; s. sabater, Universitat de Girona; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research

Rivers are subjected to multiple stressors; some of them have a natural origin like the drought, and some others are due to anthropogenic pollution, such as emerging contaminants, including pharmaceuticals. Although the effects of pharmaceutical compounds on aquatic organisms have been previously evaluated in several studies the response at community level (i.e. biofilm community), has been scarcely investigated. Besides, biological communities are often exposed to multiple stressors simultaneously. For instance, Mediterranean streams commonly have a period without flow in summer months and communities are exposed to a drought, impacting their function and structure. In this context it becomes relevant to evaluate the response of the aquatic community to both, single stressors and combination of multiple ones. Therefore, the aim of this study was to investigate the metabolic response of biofilm community exposed to a pharmaceuticals mixture (case of pharmaceuticals exposure, lipids were also the main group of compounds that can be altered by these stress factors. The experiment was carried out in artificial streams where the biofilm was exposed to a dry period (a week without flow), and to a mixture of nine pharmaceutical compounds (ibuprofen, diclofenac, carbamazepine, sulfamethoxazole, erythromycin, metoprolol, atenolol, gemfibrozil, hydrochlorothiazide) at environmental relevant concentrations. In order to classify the metabolomic profiles, a metabolomic approach was used using a liquid-chromatography coupled to mass spectrometry, LTQ-orbitrap. Results indicate a significant metabolome change in the biofilm exposed to a dry period and slight differences in the biofilm exposed to pharmaceuticals. Most of the compounds altered due to a dry period were lipids including behenic acid and lignoceric acid; but also levels of pigments like harderoporphyrin changed. In the case of pharmaceuticals exposure, lipids were also the main group of compounds identified. When both stressors were applied at the same time the response of biofilm was similar to that expressed only in the dry period pinpointing this factor as the most relevant stressor. The metabolomic approach allowed the identification of different putative compounds underlying these stress factors and belonging mainly to the lipids group that could be proposed as biomarkers of exposure.

145 Effects of global climate change on Daphnia magna metabonomics studied by GC-MS and chemometric tools

E. G. Garreta, IDAEA CSC Barcelona / Environmental Chemistry; B. Campos, IDAEA-CSIC / Environmental Chemistry; C. Barata, CSC / Environmental Chemistry; S. Lacorte, IDAEA-CSIC / Environmental Chemistry; R. Tauler, IDAEA-CSIC /

Climate change is a current concern for the scientific community, demanding novel information about the effects of environmental stressors on living organisms. Metabolomics approach tries to characterize the most relevant metabolites which have altered the internal metabolism of the organism highly used for toxicological assessment in aquatic media, although few metabolomic studies have been performed up to now. The aim of this work is to study the metabolic variation of *D. magna* individuals exposed to changes of three different abiotic factors: salinity, temperature and oxygen levels, and to achieve a tentative identification, using advanced chemometric tools. An untargeted gas chromatography method coupled to mass spectrometry (GC-MS) was developed to evaluate these effects. Polar metabolites of the whole organism were extracted by a methanol-water solution and the chromatographic separation was performed with optimization of derivatization conditions. PCA analysis of Total Ion Chromatogram has shown major changes in metabolite levels of *Daphnia* exposed to salinity and temperature. PLS-DA of the same data set together with their class assignation according to the sample treatment and Variables Importance in the Projection (VIP) scores revealed the most influent variables on the discrimination among controls and treated samples. Resolved MCR-ALS components in the analysis of the full scan GC-MS data enabled a tentative identification and confirmation of possible metabolic biomarkers of exposure. Metabolite identification through the NIST2014 database of Mass Spectra library, and an external standard, helped to confirm the altered metabolite pools caused by the studied effects in *Daphnia* metabolome. As a preliminary conclusion, the increase of salinity is shown to affect the carbohydrate and amino acid levels while temperature affected mostly amino acids. Due to the large datasets generated from full scan GC-MS analysis and to their complexity, different chemometric data analysis approaches were used to extract the information contained in the experimental data sets and to identify the most important metabolites affected by the exposure to the previously mentioned physical agents. This integrated approach allowed the resolution of the targeted complex GC-MS full scan data sets increasing the amount of information recovered and maximizing the interpretation of their biological significance.
Chemical and microbial MST tools: discrimination of faecal sources in shellfish-harvesting or bathing areas and their catchments in France

E. Jande; L. Harraud, CNRS UMR Geosciences Rennes; E. Quenet, IFREMER / Department RBE; L. Jeanneneau, CNRS UMR Geosciences Rennes; S. Lozach, IFREMER / Department RBE; P. Perigaud, CNRS UMR Geosciences Rennes; C. Marin, M. Gourmelon, IFREMER / Department RBE

The microbiological quality of shellfish-harvesting areas and bathing areas can be affected by faecal pollution from human or animal sources. In recent years, numerous microbial source tracking (MST) methods based on quantification of microbial and/or chemical compounds have been developed for differentiating between sources of contamination. Indeed, identifying and managing microbial water quality at the watershed scale is extremely challenging for effective resource management and remediation. In our study, the relevance of three host-associated real-time PCR Bacteroidales markers (HF183, Rum2Bac, and Pi2Bac) and faecal stanol as MST markers was assessed at the level of three catchments in Brittany, France. They were monitored together with fecal indicators (Escherichia coli and enterococci) and chemophysical parameters (rainfall, temperature, salinity, pH, and turbidity) by monthly sampling over 1 year (n=180 water samples). Concentrations of Escherichia coli varies from E.coli.coli>1.000 CFU/100 ml. Among the 180 water samples analysed, MST markers have assigned an origin of faecal contamination in 161 samples (89%). Host-associated Bacteroidales markers and faecal stanol assigned at least one similar origin in 109 water samples. In these 109 samples, Bacteroidales and faecal stanol assigned a bovine origin in 54% of the samples and a human origin in 39% of the samples. The porcine faecal contamination of the water has been assigned in 7% of the samples analysed. MST markers selected in this study showed that faecal contamination in water was mainly from bovine origin in these three catchments. Those results are in agreement with the landscape occupation and management. Overall results provide important information on the contamination and for hazard evaluation. These data are very useful to identify sources of pathogens such as Salmonella, Campylobacter or pathogenic E. coli which could be present in some of these water samples. This study has been partially fund by the European Regional Development Fund Interreg IVA Program.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (III)

Uptake and intracellular distribution of trivalent and hexavalent chromium in a freshwater alga

I. Aharchaou, LIEC; D.A. Vignati, CNRS / LIEC UMR; E. Battaglia, University of Lorraine LIEC CNRS UMR; F. Liu, The Hong Kong University of Science and Technology; C. Fortin, University of Quebec / Centre Eau Terre Environnement. In surface waters, chromium occurs mainly in two oxidation states, namely Cr(III) and Cr(VI). These oxidation states have different chemical features and can be individualized and tracked using different methods. While Cr(III) is known to cross the cellular membrane via the sulfate uptake pathway, the uptake mechanism of Cr(III) is not well known. Cr(III) is considered less harmful than Cr(VI) to aquatic organisms, however, the bioavailability and toxicity of Cr(III) may be underestimated. In this study, uptake experiments were performed between pH 5 and 7. Each pH, uptake rates of Cr(III) and Cr(VI) by Chlamydomonas reinhardtii were measured at different concentrations within the range 1 nM and 10 μM. In parallel, the subcellular distribution of Cr(III) and Cr(VI) in the green alga C. reinhardtii was determined in algal cells exposed for 72 h to either 100 nM Cr(III) or Cr(VI) using a homogenization protocol and a differential centrifugation approach. Results showed that the internalization rate Cr(III) increased with the concentration of Cr(III) (from 1 nM to 10 μM) and was higher at pH 7 than at pH 5 or 6. This may be partly explained by a competition between the Cr(III) and H+ for internalization sites, although we suspect that the formation of hydroxo-complexes may contribute to the overall metal uptake flux. Metal uptake profiles suggest the presence of possibly two transporters involved in the uptake of Cr(III) at pH 5 and 6 while only one was observed in the concentration range tested at pH 7. The protocols for the study of the intracellular distribution of Cr(III) and Cr(VI) by C. reinhardtii algae were developed and experiments are currently underway. Little knowledge exists about the chemical forms that control the accumulation of Cr(III) by algae, nor on the intracellular distribution of Cr in algae exposed to Cr(III) and Cr(VI). We hypothesize that Cr(III) and Cr(VI) have different fate within the cells. The subcellular distribution experiments will help to probe the detoxifying mechanisms and infer on the potential for trophic transfer of the two redox forms. Keywords: Chromium, uptake, subcellular distribution, speciation.

Algal uptake, toxicity and subcellular distribution of rare metals (Pd, Pt, La, Eu)

C. Fortin, University of Quebec / Centre Eau Terre Environnement; K. Racine, Centre d'expertise en analyse environnementale du Québec; C. Beaubien, G. Tétrault, Institut National de la Recherche Scientifique / Centre Eau Terre Environnement; S. Leguay, INRS-ETE; P.G. Campbell, Université du Québec, INRS / INRS Eau Terre Environnement.

Until very recently, little attention has been given to the ecotoxicology of platinum group elements (PGEs) and rare earth elements (REEs). Despite a recent spurt of publications on PGEs, there are still few data available for risk assessment purposes (exposure and effects). A similar lack of ecotoxicological data exists for REEs. Of particular concern is the poor solubility of lanthanides in the presence of phosphate. The toxicity of Pd and Eu on Chlorella vulgaris using the unicellular green alga Chlamydomonas reinhardtii while the toxicity of La was determined with Chlorella fusca, an alga known to accumulate enough phosphorus to sustain its growth for several days in phosphate-free media, thus avoiding the precipitation of REEs. To examine the cells’ capacity to detoxify these metals and to assess their potential for trophic transfer, we performed a subcellular partitioning scheme on metal-laden C. reinhardtii cells. This requires the determination of the cells using an ultrasonic probe followed by differential centrifugation and heat denaturation steps, resulting in five fractions: cell debris, metal-rich granules, organelles, heat-denatured cytosolic proteins (HDP), and heat-stable peptides or proteins (HSP). The most sensitive endpoint for C. reinhardtii was thus the cell population after 24 h and resulted in EC50 of 66 ± 3 nM total Pd and 26 ± 3 nM total Pt at pH 7.0. In the case of C. fusca, the final yield after five days resulted in an EC50 of 440 ± 60 nM (based on the free calculated La3+ and on the average total measured La; pH = 5.5). Finally, in order to test for the influence of speciation, several ligands were used (imidodiacetate, nitridodiacetate and malate) and showed that accumulation and toxicity can be predicted based on the free La3+ ion concentration. Clear differences were observed in the metal subcellular distribution between the “hard” (La, Eu) and “soft” (Pd, Pt) metals. They can affect metal regulation mechanisms and consequently metal accumulation patterns between populations. The between-population bioaccumulation variability is unknown and need to be assessed because it questions the relevance of biomonitoring strategies and the use of gammarids as test organisms for environmental monitoring. The aim of this study was to investigate the metal bioaccumulation abilities of different naive populations in gammarid amphipods. By this way, we sought to assess the natural range of bioaccumulation abilities between unexposed populations of gammarids. Five naive Gammarus populations from natural freshwaters exhibiting various physicochemical characteristics were collected and then exposed, in the laboratory, to various metal concentrations (Pb, Cd and Ni) for seven days following by a seven-day depuration period. The internal metal concentrations in gammarids were measured over time. Then, metal uptake and elimination rate constants describing bioaccumulation were determined for each population, using biodynamic model approach. Results show that naive populations have a generic response to metal exposures which support the ecotoxicological relevance of their use as biomonitoring tool. This generic response gives interesting perspectives towards a generic metric of gammarid as indicators of metal contamination quality. The determination of the natural variability in Gammarus and its use as reference can offer also the opportunity to assess another confounding factor affecting bioaccumulation, as chronically exposure to contamination.

Whole body metal concentrations in aquatic macroinvertebrates: mechanistic and correlative modelling

D. Hug Peter, Institut Forel, University of Geneva / Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences; V.I. Slaveykova, University of Geneva / Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences; E. Castella, University of Geneva / Institute Forel Earth and Environmental Sciences and Institute of Environmental Sciences

Whole body metal concentrations in aquatic macroinvertebrates convey a valuable starting point to investigate the link between environmental conditions, uptake and toxicity. Despite advances in the understanding of uptake and bioavailability, they...
remain hard to interpret and hard to predict from environmental conditions. In this study, field data was collected for two different approaches: 1. test the ability of existing biometric parameters to predict whole body metal concentrations in aquatic macroinvertebrates in model species and closely related taxa; and 2. develop a multiple regression model to predict metal accumulation in macroinvertebrates from concentrations in water and sediment. Whole body metal concentrations of a broad range of aquatic macroinvertebrates and concentrations of metals in water, sediment and suspended particulate matter of all sites were used in both models. Whole body concentrations estimated with the biodynamic model were within the measured range of concentrations for Cd in *L. stagnalis* and *D. polymorpha* For Cu, values were systematically underestimated. When transferred to other gastropod species, the *L. stagnalis* model predicted whole body concentrations for Cd in the same order of magnitude as measured concentrations but the accuracy of the results was poor. Preliminary results of the correlational approach were promising. The models for different metals were able to explain 25 to 70 % of the variance (adjusted R²). The significant parameters conveyed information on different uptake mechanisms. Preliminary prediction trials showed that the influence of environmental conditions and of different types of taxa needs to be tested further in order to predict Cu, in *C. sikamea*, the contrast in metal speciation between normal and contaminated individuals was similar to but much less pronounced than that in *C. hongkongensis*. Cu and Zn occurred mainly in species resembling cysteine complexes; in the gill of contaminated ones, the major metal species resembled histidine complexes or phosphates. Speciation of Cu and Zn in mantle was similar to that in gill, both of which were different from that in digestive gland. Even in the Digestive gland of heavily contaminated *C. hongkongensis*, the proportion of thiocysteine species was only about 10% of Cu species. In contrast, the contrast in metal speciation between normal and contaminated individuals was similar to but much less pronounced than that in *C. hongkongensis*. Cu existed in different oxidation state when bonded with different atoms, i.e., as Cu(I) when bonded with sulfur, and as Cu(II) when bonded with oxygen or nitrogen. This study provided direct and semi-quantitative information on the changes of metal speciation in contaminated oysters, indicating that in metal-rich environments oysters could efficiently detoxify excessively accumulated Cu and Zn by storing them in oxygen- and nitrogen-bonded complexes.

152 Response of *Crassostrea rhizophorae* to metal and metalloid environmental contamination: Integrating Chemical and Biological Data by Chemometrics Ld. Souza, Ciencias Fisiológicas; M. Morozeski, UFSCar / Departamento de Ciências Fisiológicas; M. Bonomo, Universidade Federal do Espírito Santo / Departamento de Ciências Biológicas; V. Azevedo, Universidade Federal de São Carlos; M. Monferran, Universidad Nacional de Cordoba; S. Matsumoto, C. I.d. Souza, Universidad Nacional de Cordoba / Ciencias Fisiológicas.

In this study, chemical and physical parameters were analyzed in two Brazilian estuaries, Vitoria bay (sites: Santa Maria, Serra and Lameirão) and Santa Cruz bay (sites: Santa Cruz). In order to assess the bioavailability of metals and their accumulation in the soft tissue of the oyster *Crassostrea rhizophorae*. Levels of B, Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Pb and Hg in surface water and oyster soft tissue were correlated with exposure and biochemical biomarkers and histopathological change indexes in the gills. The use of physical and chemical analysis integrated with biomarkers provided cause and effect data for the determination of metal contamination and its effect on the resident organisms. The GPA analysis allowed the observation of a seasonal difference in Santa Maria and the rain influence in areas with high riverine input. This method, when integrated with biological data showed a high efficacy in area differentiation, showing that biological alterations are correlated with environmental physical and chemical conditions. In addition, the biochemical mechanisms were effective in metal elimination, especially metallothionein, while the oyster morphology was affected mainly by environmental data, such as salinity. Keywords: estuary, histopathology, oyster, antioxidant system.

**Emerging contaminants of urban origin: sources, sampling, analytical issues and remediation techniques (III)**

153 Bismuth in cosmetic products and its implications for sewage sludge management J. Amnekeley, L. Sorren, Statistics Sweden / Environment and tourism; A. Augustsson, Linnaeus University; B. Bergback, Linnaeus University / School of Natural Sciences.

Bismuth (Bi) is a heavy metal that over recent years has shown increasing concentrations in sewage sludge in Swedish wastewater treatment plants (WWTPs), indicating an increasing Bi use in the society. The high accumulation rate of Bi in soil when sewage sludge is used as fertilizer on arable land is of environmental concern. Bismuth is used in various consumer products to replace lead, but which sources in the society that explain the increasing amount of Bi in sewage sludge in the municipal WWTPs is however unknown. This study aimed to analyze one product group suspected to contain Bi, cosmetics, and three different products were chosen (foundation, powder and eye shadow) in order to study the flows in urban wastewater in Stockholm, Sweden. The chemical analyses showed that Bi was present in very high concentrations (>100 000 mg/kg) in one third of the analyzed foundation and powder samples, while mainly low concentrations were found in eye shadow. These cosmetic products explained approximately 24 % of the measured total Bi amounts reaching the WWTP in 2012, making cosmetics a major Bi source. It is therefore recommended to decrease the emission of Bi to WWTPs and to decrease the emission from Bi in cosmetics to the WWTPs.

154 The first application of wastewater-based drug epidemiology in five South Korean cities K. Kim, Department of Civil and Environmental Engineering; F.Y. Lai, Entox / National Research Centre for Environmental Toxicology Entox; H. Kim, Pusan National University / Civil and Environmental Engineering; P.K. Thai, J. Mueller, Entox / National Research Centre for Environmental Toxicology Entox; J. Oh, Pusan National University / Department of Civil and Environmental Engineering.

Illicit drug consumption in five cities in South Korea was estimated by analyzing 17 drug residues in untreated wastewater samples that were collected during the Christmas and New Year 2012–13 holidays. Only methamphetamin, amphetamine, and cocaine were detected at concentrations of tens of nanograms per liter or even lower concentrations in more than 90% of the samples. Other illicit drugs were detected in very low concentration (including cocaine, methadone, and benzoylecgonine) and have not been detected frequently in wastewater from other countries were not found in this study. Methamphetamine was found to be the most widely used illicit drug in South Korea, and the estimated average consumption rate was 22 mg d⁻¹ (1000 people) °. This rate is, for example, 2–5 times lower than the estimated average concentration rates in Hong Kong and other parts of China and 4–80 times lower than the estimated average consumption rates in Indonesia and the Netherlands. Based on the assumption that the measured concentrations were representative of the survey period and catchments of this study are representative of South Korea all year round, the available extrapolation suggests an annual consumption of methamphetamin of about 148kg.
samplers. The measured concentrations of the CECS from this study were lower than previously published data on the levels of the target compounds in treated effluents from other Canadian WWTPs. Artificial sweeteners, sacralose and acesulfame are persistent compounds that were present at relatively high concentrations in effluents at all sampling sites. Concentrations of carabazinepine and trimethoprim were estimated to be between 6 to 70 ng/mL. Overall, these data indicate that the treatment design is not able to improve wastewater quality for conventional parameter (e.g. phosphorus) reduce, but do not entirely eliminate CEC inputs in the Lake Simcoe watershed. Keywords (4 max): Wastewater, passive samplers, pharmaceuticals, personal care products

156 Predicted environmental concentrations of cytostatic drugs in Catalonia (NE Spain), and comparison to measured concentrations in Besós River
H. Franquet, Environmental Chemistry; C. Gómez-Canela, F. Ventura, J. Caixach, IDAEA-CSIC; S. Lacorte, IDAEA-CSIC / Environmental Chemistry

The incidence of cancer in Catalonia has increased considerably in the last years, with 33715 cases registered in 2007. Cancer is treated with cytostatic drugs which can be excreted unchanged or modified as metabolites and be discharged into the sewer system. Besides, elimination of these drugs in waste water treatment plants (WWTPs) is often incomplete [1] and they can finally reach surface waters. The aim of this work was to study the consumption of all the cytostatic drugs used in Catalonia in the period 2010-2012 in order to calculate the predicted environmental concentrations (PECs). PECs are used to estimate the amounts of drugs expected to be discharged into the environment using publicly available consumption data, excretion values and wastewater elimination rates. Two out of 132 cytostatics had PEC values higher than 10 ng L~1~ (mycophenolic acid and hydroxyurea), which is the EMEA threshold for environmental risk assessment [2]. Considering PECs and ecotoxicological data available, the risk quotient was calculated to determine the potential adverse effects of cytostatics in the environment. The results showed no risk for the aquatic environment. Currently, ecotoxicological data is scarce and only individual drugs toxicity is reported in the literature. In a second step, 25 cytostatic drugs, which had the highest PEC values, were analyzed in water samples collected along the Besós River basin, bordering the city of Barcelona and receiving WWTP discharges of 25 plants. Samples were extracted using Oasis HLB cartridges and eluted with acidified methanol. An LC-HRMS Orbitrap under positive ESI mode and a Luna C18 column were used to perform the analysis. Fifteen drugs were detected, being mycophenolic acid, ifosfamide and chlorambucil the most prevalent compounds. The results obtained were compared with the previously calculated PECs to validate the procedure. Together with *Daphnia magna* EC~50~ values, the risk of cytostatic compounds in the aquatic environment was assessed. 1. Heredia, M.; Puyo, M.; Prat, M.; Burgués, M.; Pujadas, J. M.; Lloret, M. *Environmental, fate and human exposure to veterinary medicines and pharmaceutical residues in the aquatic environment: A Review of recent research data.* Toxicology Letters. 2002. 131(1-2): p. 5-17. 2. EMEA, *Guideline on the environmental risk assessment of medicinal products for human use.* 2006.

157 Stormwater in Saskatoon - Stormwater in Creysmore Conditions
G. Codling, University of Saskatchewan / Toxicology; H. Yuan, University of Saskatchewan / Toxicology Centre: M. Hecker, University of Saskatchewan / School of the Environment and Sustainability and Toxicology Centre; P.D. Jones, University of Saskatchewan / School of Environment and Sustainability; J.P. Giesy, University of Saskatchewan / Toxicology Centre

Water samples were extracted and cleaned up using solid phase extraction. A PFAA concentration in atmospheric deposition (rain and snow) as well as two rivers from watersheds with varying catchment area and hydrological function. The 14 target PFAAs (project CGL2013-4 PSR) were measured in the south Saskatchewan River as well as the water transit time between atmospheric deposition and riverine outflow in combination with emission changes over time alter the PFAA pattern within the catchments.

**POPs and Pseudo-POPs: Environmental occurrence and exposure (III)**

159 A seasonal atmospheric assessment of POPs and pseudo-POPs using passive sampling and modelling
P. Jiménez-Guerrero, University of Murcia / Physics; S. Ramos, University of Porto (LEPAEDECOPEUP); I. A. Silva, LEPAE University of Porto; A. Alves, Faculty of Engineering - University of Porto; J.P. Montávez, Department of Physics University of Murcia; N. Rato, LEPAE- University of Porto / Physics of the Earth

The atmosphere is a fundamental route of transport for numerous contaminants, so it is very important to measure their levels in the air. And even if some effects are already reported in literature, there are still numerous regions where the information is scarce, like the southern part of Spain in Catalonia. With specific climatic fingerprints, it can be an excellent location to assess the atmospheric distribution of contaminants, on a seasonal perspective. Passive air samples (PAS) have been recently used at regional, continental and global scales and their feasibility was demonstrated in deployments across large areas. Polyurethane foam (PUF) disks were deployed sequentially every three months for a period of one year in 13 sites, comprising four sampling campaigns, one per season. Five classes of POPs as well as POPs were analysed: three belonging to the persistent organic pollutant (POPs) group - brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs, represented by HCB), and two other which can be considered pseudo-POPs - polycyclic aromatic hydrocarbons (PAHs) and synthetic fragrances (musk). The main objective of this work was to assess the levels and the spatial and temporal patterns of these pollutants in the Levantine coast (south-east Iberian Peninsula). Atmospheric transport features were also investigated, in correlation with the meteorological data, showing a decisive influence of these parameters. Field data and CTMs were combined to produce a comprehensive overview of a region with a lack of information on these pollutants. Higher levels for PAHs were observed in summer (autumn/summer) while lower levels were found in the warmer ones (spring/summer). The same was observed for OCPs. In the case of PCBS and BFRs, the predominance was usually found in the spring/summer seasons. Acknowledgements: This work has been partially funded by the European Union 7th Framework Programme-Marie Curie Cofund (FP7/2007-2013) under contract 2617143 and by the Operational Programme for Competitiveness Factors - COMPETE and National Funds through FCT (Fundação para a Ciência e a Tecnologia) under the NORTE-07-0124/FEDER-000025. The Spanish Ministry of Economy and FEDER (project CGL2013-48491-R) are acknowledged. Dr. Pedro Jiménez-Guerrero acknowledges the Ramón y Cajal programme.

160 Persistent organic pollutants in background air in central Europe - parameters affecting wet scavenging of polycyclic aromatic hydrocarbons
P. Shahpouri, Max Planck Institute for Chemistry / Multiphase Chemistry; G. Lammel, Masaryk University; A. Holubová-Smečkalová, Czech Hydrometeorological Institute; I. Klimova, Masaryk University; M. Váňa, Czech Hydrometeorological Institute

Currently an increasing number of studies have reported on the occurrence of POPs in seemingly pristine groundwater and rivers worldwide. However, there are no studies which have evaluated how PAHs enter surface water from pristine terrestrial ecosystems. The aim of this study was to investigate the transport of PAHs from atmospheric deposition into surface water in a pristine terrestrial environment. The study was conducted within a low population, uniquely instrumented research catchment in Northern Sweden. The work included measuring PAH concentrations in atmospheric deposition (rain and snow) as well as two rivers from watersheds with varying catchment area and hydrological function. The 14 target PAHs (project CGL2013-4 PSR) were measured in the south Saskatchewan River as well as the water transit time between atmospheric deposition and riverine outflow in combination with emission changes over time alter the PFAA pattern within the catchments.
particulate matter and rainwater properties were investigated. The concentrations of ionic species in particulate matter and rainwater were significantly correlated, highlighting the importance of particle scavenging process. Overall, higher scavenging efficiencies were found for relatively less volatile PAHs, underlining the effect of analyte gas-particle partitioning on scavenging process. The PAH wet scavenging was more effective when the concentrations of ionic species were high. In general, the concentration of PAHs and ionic compounds in the particulate matter were found to influence the PAH scavenging. Keywords: gas-particle partitioning, ionic species, elemental carbon, organic carbon

161 Passive sampling of PAHs on the petrochemical area of Tarragona County, Spain: PUFs vs. vegetation

N. Domínguez, URV/ISPV; L. Vilavert, Universitat Rovira i Virgili; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering; J.L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; M. Nadal, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health

Since 2002, the environmental concentrations of polycyclic aromatic hydrocarbons (PAHs) are periodically assessed in Tarragona County, Spain, where the most important chemical/petrochemical industrial complex in Southern Europe is located. The aim of this study was to determine the levels of 16 PAHs in air and vegetation of different areas of Tarragona County, Spain, as two methods of passive sampling. Temporal trends of PAH levels in vegetation were estimated by comparing with results from previous surveys. Passive air samplers (PAS) with polyurethane foam (PUF) were used for air monitoring (Newterra, Beamsville, ON, Canada). In June of 2014, 27 vegetation samples were collected in different zones of Tarragona County. In parallel, PUF-PAS were deployed at 16 sampling points in Tarragona County for a period of 2 months. Four samples were collected in each one of the areas under study (petrochemical, chemical, urban/residential, and unpolluted). The analysis of the extract BAS was 21.4, 29.8, 26.5 and 29.4 ng/m² in the petrochemical, chemical, residential/urban and unpolluted areas, respectively. Ranges of the 16PAHs concentrations were similar, regardless of the sampling area, with a maximum value of 55.8 ng/m² in the industrial zone. Airborne levels were very similar to those previously reported in a number of countries. No significant differences between the study areas were found (p>0.05), likely associated with the sample loss due to the high temperatures in the summer season. The average concentration of 16 PAHs in vegetation was 66.6, 194, 229 and 145 µg/kg in the petrochemical, chemical, residential/urban and unpolluted areas, respectively. A general increase of the levels of 16 PAHs in vegetation can be seen, if compared with data from the first survey, performed in 2002. No significant differences between the study areas were found, with the exception with the raise found in the unpolluted area. Finally, if we compare the PAHs concentrations reported by both monitors, trends are similar in the different sampling areas. The current concentrations of PAHs in samples of ambient air and vegetation of Tarragona are very similar to levels reported in the scientific literature. These results will be used to design a new monitoring network for assessing the occurrence of these pollutants in Tarragona County, an area highly impacted by the petrochemical industry.

162 Long-term environmental monitoring of PCDD/Fs near a municipal solid waste incinerator in Tarragona County (Catáliolania, Spain)

J.M. Sardà, Universitat Rovira i Virgili; L. Vila, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering; J.L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health

For approximately 20 years, the concentrations of polychlorinated dibenz-p-dioxins and dibenzofurans (PCDD/Fs) have been periodically measured in soil and vegetation samples from its surroundings. Since 2006, air samples are also collected by means active and passive sampling devices, and PCDD/F levels are determined [1,2]. The first aim of this study was to determine the current environmental status in the MSWI vicinities by analyzing PCDD/F levels in soil, vegetation and air samples collected between 2013 and 2014. Secondly, the temporal trends of the study areas were found, with the exception with the raise found in the unpolluted area. In May/June of 2013, 8 vegetation and 8 air samples were collected around the facility. The sampling sites were the same as those corresponding to the baseline and subsequent surveys. In June of 2014, 8 soil and 8 air samples were collected at the same sampling points. The concentrations of PCDD/Fs in environmental samples were determined by means of high resolutions gas chromatography coupled to high resolution mass spectrometry (HRGC/HRMS). The concentration of PCDD/Fs in eight soil samples varied between 0.16 and 1.61 ng WHO-TEQ/kg dry weight (dw), with an average of 0.63 ng WHO-TEQ/kg (dw). It meant a non-significant decrease comparing to the baseline study (1999) of 47%, and a slight increase to the previous study (2010) of 9% (p<0.05). The concentration of PCDD/Fs in vegetation, analyzed in 2013, ranged from 0.04 to 0.10 ng WHO-TEQ/kg (dw), with an average of 0.07 ng WHO-TEQ/kg (dw). A general increase of the levels of 16 PAHs in vegetation can be seen, if compared with data from the first survey, performed in 2002. No significant differences between the study areas were found, with the exception with the raise found in the unpolluted area. Finally, if we compare the PAHs concentrations reported by both monitors, trends are similar in the different sampling areas. The current concentrations of PAHs in samples of ambient air and vegetation of Tarragona are very similar to levels reported in the scientific literature. These results will be used to design a new monitoring network for assessing the occurrence of these pollutants in Tarragona County, an area highly impacted by the petrochemical industry.

163 Air-Water Exchange of Natural Brominated Compounds in the Northern Baltic Sea: New Estimates and Possible Influences of Climate Change

T.F. Bidleman, Umea University / Chemistry; K. Agosta, Umea University; A. Andersson, Umea University / Ecology and Environmental Science; P. Haglund, Department of Chemistry; A. Hegmans, Royal Roads University / Environmental Science; L.M. Jantunen, Environment Canada; O. Nygren, Umea University / Department of Chemistry; J.O. Okeme, University of Toronto / Physical and Environmental Sciences; J.L. Vila, University of Waterloos / Chemistry; M. Ripszam, M. Tysklind, Umea University / Department of Chemistry

Bromophenol (BPs) are produced by macroalgae and cyanobacteria in the Baltic Sea and oceans worldwide. Compounds derived from BPs include bromoaromatics (BAs), hydroxylated and methoxybrominated methylphenyl ethers (OH-BDEs, MeO-BDEs) and polybrominated dibenz-p-dioxins (PBDDs). Although natural, these compounds have toxic properties which may be bioavailable when viewed as part of the mixture with anthropogenic substances. Here we update air-sea exchange fluxes of BAs in the subarctic Gulf of Bothnia, report the first measurement of MeO-BDEs in Baltic water and air, and discuss how climate change may influence production of natural brominated compounds. Water was passed through glass fibre filters (GF/F) then through columns of XAD-2 or ENV+ (Fisher Scientific, Fair Lawn, NJ, USA). Samps were collected on GF/F filters. Tetrabrominated MeO-BDEs were confirmed by GC high resolution mass spectrometry. Henry’s law constants (HLCs) for 2,4-DiBøa and 2,4,6-TrBa were determined in deionised water from 5–30°C using the bubble stripping method. The most abundant BAs in water were 2,4,6-TrBa and 2,4-DiBa at 64–637 and 20–205 pg L⁻¹, while these BAs were in air from 5.5–116 and 2.3-37 pg m⁻³. MeO-BDE47 and 2′-MeO-BDE68 were found in Baltic water (5-50 and < 3–25 pg L⁻¹) and air (11–43 and < 1 pg L⁻¹). New gas exchange fluxes of BAs were calculated with the two-box model using temperature-dependent HLCs determined here. Updated 2,4-DiBa + 2,4,6-TrBa exchange from Bothnian Bay (38000 km²) between May and September was 650 kg, compared to our previous estimate of 1310 kg. Impacts of climate change on the Baltic Sea include increasing water temperature, greater discharge of fresh water and dissolved organic matter (DOM), and reduction in ice coverage. A shift in species may have been due to change in water-based food web in the northern Baltic, whereas phytoplankton production in the south is expected to increase under some scenarios of rising temperature and nutrient loadings. These factors may favour or oppose formation of halogenated natural products, but their collective effect is far less clear. A better understanding of their biogeochemistry is required!

164 From surroundings to our clothing: Determining uptake rates of fabrics for SVOCs in indoor environment.

A. Saini, University of Toronto / Department of Physical and Environmental Sciences; J.O. Okeme, University of Toronto / Physical and Environmental Sciences; M.L. Diamond, University of Toronto / Department of Earth Sciences

Fabric used in clothing, upholstery, linens and many more applications vary in chemistry as comprised of natural to various synthetic fibres. Clothing can sorb VOCs or SVOCs from the surrounding environment and release them after removal of the source (De Coensel et al., 2008; Feldman, 2010). Chemical sorption by fabric can influence the rate of human exposure even after pesticide spraying through direct transfer to skin or inhalation of gas-phase pesticides that were released to personal cloud. Horstmann and McLaughlan (1994) reported the contaminated T-shirts as source of PCDD/F to human epidermis in wearing trials. The transfer of the compounds from the T-shirt fabric to skin indicated that the chemicals are reversibly sorbed to the fabric. Thus, it stands to reason that SVOCs such as flame retardants and plasticizers, will partition to clothing according to the physical-chemical properties of the SVOCs as well as fabric. The aim of this study was to determine the uptake rates of SVOCs for fabrics and to understand sorption behaviour. Our results reveals that both cotton and rayon show linear uptake pattern of flame retardants and penta-BDEs for more than a month during deployment period, for instance, uptake BDE-47 at the rate of 2.8 pg cm⁻² of fabric. Cotton and rayon which is “regenerated” cellulose fabric (Shaikh et al, 2012), showed similar sorptive behaviour. Also, fabric (cotton)-air partitioning coefficients measured for NFRs and BDEs shows good
of possible genotoxic mechanisms in the absence of ROS production. PLHC-1 cells are a good model for ecotoxicology studies of AgNP to be used in the framework of intelligent testing strategies. Acknowledgements - This research was supported by FP7-MARINA project (Grant Agreement No. 263215).

167 Toxic effect of pristine SWCNT combined with Cadmium to the crustacean Daphnia magna 
M. Rwey, INRS-Institut Armand-Frappier; M. Fournier, Institut Armand Frappier-INRS / Immunotoxicology Laboratory; P. Robidoux, Université de Sherbrooke / Aquatic and Crop Resource Development Portfolio Production increase of carbon nanomaterials and development applications raises the question of the consequences of their release on wildlife, including pelagic species which may be exposed. In Canada, the forestry industry experienced an economic crisis which has led to the elaboration of new forestry products like nanomaterials, including single-walled carbon nanotubes (SWCNT). SWCNT offers many benefits because of their high electrical and thermal conductivity and also mechanical properties such as toughness and strength. On top of that, they possess a very large surface area allowing them to adsorbed hydrocarbons or metals present in water or soil. Consequently, they are closely studied for environmental monitoring and wastewater treatment. Nowadays, It’s important to study the potential interactions between carbon nanomaterials and environmental contaminants. This study focuses on the toxicity of single-walled carbon nanotubes (SWCNT) in presence of cadmium (Cd), using the crustacean Daphnia magna and 48 h acute test. The LC50 for Cd or pristine SWCNT is also as their antioxidant by using the protective effects of nanoceria in certain neurological and cardiological disorders; seems to provide cells protection from radiation. However, CNPs have also been found to display oxidase activities and to generate damaging oxygen radicals in a range of organisms and cell systems. We have performed a thorough study of the effect of different CNPs on a model aquatic microorganism, the green alga Chlorella vulgaris, and that the presence of SWCNT increases the toxicity of Cd to D. magna. LC50 for Cd and SWCNT were 252.3 and 1400 µg/L, respectively. In the presence of a fixed concentration of SWCNT, LC50 of Cd decreased. The lowest LC50 of Cd was 111.1 µg/L in presence of 1000 µg/L of SWCNT, indicating an increase in Cd toxicity. SWCNT accumulated in the digestive tract of D. magna was confirmed by binocular microscope observations. The increase in toxicity may be due to the adsorption of Cd on the carbon nanotubes which induces a higher entrance of Cd in the digestive tract of D. magna. Then, once entered into the digestive tract, Cd will be desorbed helped by the enzymatic components contributing to desorption processes. Those results show the complexity of SWCNT toxicity and how the understanding of their interactions with other contaminants is crucial to determine the consequences of their release in the environment.

168 The effect of colloidal stability and Ce valence states at the surface of cerium oxide nanoparticles on cellular toxicity
G.P. Reyes, Biology department: I. Rodea-Palomares, F. Leganes, Universitat Autònoma de Madrid / Biology, S. Das, S. Seal, University of Central Florida; R. Rosal, Ingeniería Química; F. Fernandez-Piñas, Universitat Autònoma de Madrid / Biology Cerium oxide nanoparticles (CNPs) have been used in numerous engineering and biomedical applications: in plants to increase photosynthesis and biochemical sensing. Nat Mater 8: 1004-1010; seems to provide cell protection from radiation. However, CNPs have also been found to display oxidase activities and to generate damaging oxygen radicals in a range of organisms and cell systems. We have performed a thorough study of the effect of different CNPs on a model aquatic microorganism, the green alga Chlorella vulgaris, and that the presence of SWCNT increases the toxicity of Cd to D. magna. LC50 for Cd and SWCNT were 252.3 and 1400 µg/L, respectively. In the presence of a fixed concentration of SWCNT, LC50 of Cd decreased. The lowest LC50 of Cd was 111.1 µg/L in presence of 1000 µg/L of SWCNT, indicating an increase in Cd toxicity. SWCNT accumulated in the digestive tract of D. magna was confirmed by binocular microscope observations. The increase in toxicity may be due to the adsorption of Cd on the carbon nanotubes which induces a higher entrance of Cd in the digestive tract of D. magna. Then, once entered into the digestive tract, Cd will be desorbed helped by the enzymatic components contributing to desorption processes. Those results show the complexity of SWCNT toxicity and how the understanding of their interactions with other contaminants is crucial to determine the consequences of their release in the environment.

169 Toxicity and dietary transfer of silver nanoparticles

Biophysical Interactions at the Bio-nano Interface: Relevance for Aquatic Nanotoxicology

The zebrasharp embryo chorion as a biological barrier for nanoparticles uptake?
S. Bohm, Bioanalytical Ecotoxicology; M. Baccaro, UFZ Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology; M. Schmidt, UFZ Helmholtz Centre for Environmental Research / Isotope Biogeochemistry; H. Stäck, UFZ Helmholtz Centre for Environmental Research / Analytical Chemistry; T. Riehme, Helmholtz Centre for Environmental Health; D. Kühn, Helmholtz-Centre for Environmental Research / Bioanalytical Ecotoxicology

The zebrasfish (Danio rerio) embryo is an established test organism to investigate the toxic potential of chemicals and can be adapted to test nanomaterials by minor adjustments. Compared to chemicals and due to the individual chemical and physical properties of nanomaterials a different and more complex interaction of nanomaterials and the biological component of the zebrasfish embryo can be assumed. Especially, the embryo chorion as a possible biological barrier is discussed and was investigated in detail in this study. The investigated silver, gold, and copper nanoparticles were provided and characterised by partners of the EU-project NanoValid. Time kinetic experiments were conducted and the concentration associated to different compartments (the whole egg (embryo with intact chorion) and the embryo) were determined by acid digestion and ICP-MS measurement of the received homogenous samples. For all types of particles a time-dependent concentration increase within the chorion and for the whole organism can be observed. The element concentration obtained for the embryos is almost constant over time, independent of the type of particle. In addition, the visualization of the particle distribution by means of LA-ICP-MS was performed. Locate and particle agglomerates were identified. Ag was detected, but it was not possible to observe particles or particle agglomerates probably due to the high solubility of this type of particles. Finally, a sorption model based on particle number calculations necessary to saturate the surface and to form particle layers on the chorion was proposed.
the viewpoint of agricultural water scarcity. Characterisation factors (CFs) at the country scale were developed to model the impact of food trade links food exporter and importer on the grey water footprint. The characterization model includes three parts: 1) food supply chain assessment and 3) health damage assessment) was developed to model the impact of food trade links food exporter and importer on the grey water footprint. The characterization model includes three parts: 1) food supply chain assessment and 3) health damage assessment.)

Advancements in life cycle impact assessment and footprint method development (III)

170 Human health impact assessment model considering trade-induced effects arisen from agricultural water scarcity

M. Motoshita, National Institute of Advanced Industrial Sci. and; Y. Ono, S. Pfister, ETH Zurich; A. Boulay, CIRAIG - École Polytechnique de Montréal / Chemical engineering department; M. Berger, Technische Universität Berlin / Chair of Sustainable Engineering Office Z; K. Nansai, Nat. Inst. of Env. Studies (NIES); K. Tahara, National Institute of Advanced Industrial Science and Technology / Research Institute of Science for Safety and Sustainability; N. Isubo, Tokyo City University; A. INABA, Kogakuin University; M. Finkbeiner, DaimlerChrysler AG / Chair of Sustainable Engineering

Assessing the potential impacts of water use has become one of the great concerns in life cycle assessment. Especially, demand for agricultural use dominates a large part of total water demand for human society and will be significantly affected by water consumption patterns. Introducing water scarcity into the characterization module allows us to evaluate the trade-off between food quality and human health damage. In this study, we used a model to evaluate the potential impacts of water use on human health. The model predicted steady-state ag concentrations in D. magna are unlikely to cause adverse effect. Given that daphnids did not eliminate completely Ag NPs, there is a possibility of transfer of Ag NPs along the food chain.

172 A methodology and model for characterizing regional and temporal impacts of power plant thermal emissions on rivers

P. Van Sprang, School of Life Sciences; K.B. Paul, Heriot Watt University / SLS; F.R. Khan, Roskilde University / ENSPAC, V. Stone, Heriot Watt University; T.F. Fernandes, Heriot-Watt University / School of Life Sciences

The objectives of this study were to investigate the toxicity and dietary transfer of AgNO₃ and Ag NPs with different coatings (PVP, PEG and Citrate) using two freshwater organisms, the green alga, Chlorella vulgaris and the crustacean, Daphnia magna. The toxicity effect in C. vulgaris (IC₅₀ of 5.3 ± 0.5 µg L⁻¹) to C. magna was significantly higher than the Ag NPs. Citrate and PEG-coated Ag NPs exhibited similar toxicity (IC₅₀ values of 9.2 ± 1.0 and 9.3 ± 0.1 µg L⁻¹, respectively), while PEG-Ag NP was significantly less toxic to the algae (IC₅₀ of 49.3 ± 5.2 µg L⁻¹). Uptake rate constant (K_u) and membrane transport characteristics (B_max, K_g and Log K) were determined after exposing algae to ranges of concentrations of AgNO₃ or Ag NPs. The K_u and Log K correlated well with the toxicity in the algae. Transmission Electron Microscopy was used to observe possible internalization of Ag NPs in algal cells after exposure to low concentrations for 72 h (mimicking inhibiton tests) or to high concentrations for 4 h (mimicking dietary exposure). Ag NPs were visualized only in algal cells used in the dietary exposure, probably due to the increased membrane permeability of C. vulgaris. To establish biodynamic model parameters (i.e. assimilation efficiency and influx rate constant), D. magna were fed Ag-contaminated algae and depurated for 96 h. The two compartmental loss of Ag showed that D. magna assimilated significantly less Ag from AgNO₃-exposed algae (8%) than from the Ag NP - exposed diet (14-16%). However, PVP-, and PEG-coated Ag NPs were more difficult to eliminate by the daphnids. Biodynamic model prediction revealed that food is the dominant pathway of Ag uptake in D. magna, except for AgNO₃ when applying the low Ag concentration in food. Model-predicted steady-state Ag concentrations in D. magna are unlikely to cause adverse effect. Given that daphnids did not eliminate completely Ag NPs, there is a possibility of transfer of Ag NPs along the food chain.

Evaluating the potential impacts of water use has become one of the great concerns in life cycle assessment. Especially, demand for agricultural use dominates a large part of total water demand for human society and will be significantly affected by water consumption patterns. Introducing water scarcity into the characterization module allows us to evaluate the trade-off between food quality and human health damage. In this study, we used a model to evaluate the potential impacts of water use on human health. The model predicted steady-state ag concentrations in D. magna are unlikely to cause adverse effect. Given that daphnids did not eliminate completely Ag NPs, there is a possibility of transfer of Ag NPs along the food chain.
LCA of complex systems: delusion or reality? (II)

175 Guiding development of interconnected bioenergy and bioproduct systems with life-cycle energy, water, land, and greenhouse gas metrics

C.D. Scown, Lawrence Berkeley National Laboratory / Environmental Energy Technologies Division; A. Horvath; T.E. McKenzie, University of California / School of Public Health

Bioenergy is likely to be an important component of many countries’ future renewable energy portfolios. Liquid biofuels are particularly critical to providing near-term carbon mitigation without relying on the slow turnover of fleet vehicles and construction of new infrastructure. However, bioenergy production systems exist at the intersection of biology, ecology, chemistry, engineering, and policy, which makes optimizing these systems a complex and interdisciplinary challenge. We present the results of a long-term research effort aimed at quantifying the carbon, energy, water, and economic tradeoffs of advanced biofuel production strategies. First, we examine the challenge of producing ethanol from cellulose-rich feedstocks in the United States, including Miscanthus x giganteus, corn stover, and wheat straw. A 40 billion l/year scenario shows that the land footprint of dedicated crops can be minimized by selecting land with the largest yield/harvest difference between the incumbent feedstock (corn) and Miscanthus, totaling 7.5 million ha. Eliminating drought-prone counties and constraining conversion to regions with the largest carbon offset credit for exported power increases total land requirements to 8.8 million ha; in both cases, short-term net carbon sequestration could reach 25 GtCO2/My due to power offset credits and soil carbon sequestration. Adding corn stover and wheat straw that can be sustainably harvested and economically transported to commercial-scale biofuel refineries allows total cellulosic ethanol production to reach 60 billion l/year. Because the modeled biofuel locations are aligned with U.S. coal-fired power plants, we show that bioenergy capital costs can be substantially reduced, life-cycle water impacts can be reduced, and greenhouse gas (GHG) emissions can be held constant by exporting lignin for production of a variety of materials, including flame retardants and other bio-products.

176 Does resilient mean eco-efficient?

M. Pirzeg, Aalborg University / Development and Planning

Resilience is a property of systems that is deemed essential for their sustainability. The term “resilience” has become a vague buzzword in the last few years but the literature agrees that resilience depends on the structure and architecture of a system. It increases with system complexity, because the redundant connections between elements of a system make it less efficient but also more flexible and adaptable to perform. Function even if some connections are interrupted or missing. Balancing between resilience and efficiency seems to be the key for sustainability intended as long-term performance. Resilience is not explicitly taken into account within life cycle assessment (LCA). LCA determines the eco-efficiency of product systems, i.e. the ratio between the function provided by the product and its impact on the environment. The question is whether a product system in which structure is improved or designed to be more resilient will not only be more efficient, but also eco-inefficient, when studied by means of LCA. In this work a two steps approach is proposed to study resilience of product systems: 1) assessment of disturbance conditions and their inclusion within the scope of the LCA study; 2), system expansion, i.e. changing the structure of the system by including additional disturbance-preventing processes and disturbance-dependent ones. This theoretical LCA modelling approach was tested on two fictional case studies using data from literature: A) the example of two different waste management systems of a remote island disturbed by stormy weather; B) the example of two different biofuel systems, single- and multiple-feedstock, disturbed by climate changes. Results show that if proper LCA modelling is applied, resilient product systems are not necessarily less eco-efficient than their vulnerable counterparts and instead can allow for eco-efficiency gains. This goes against the intuitive idea that optimizing a system for efficiency only will necessarily allow achieving eco-efficiency as well, and suggests that design for resilience may be a valuable idea towards sustainability. However, further experimental work on the subject is needed to draw more robust and generalizable conclusions.

177 Implementation of recycling systems: The delusive role of LCA

T. Kage, F. Dinkel, Carbotech AG

Shall materials with heating values, from a demand point of view, be materially recycled or rather thermally utilised in order to get the highest benefit? This and similar questions are often raised by governments and politics. We realised that the results strongly depend on the respective allocation approach. For instance, the benefit of incineration increases when the proportion of fossil fuel in the energy mix it replaces is higher. In some cases this benefit can be high enough for thermal utilisation to seem to be, from an environmental perspective, a better option than material recycling. In other words: in countries with poor energy strategies, recycling of materials with heating values would be less advisable. On the other hand, material recycling is highly profitable in case a region only uses renewables. Hence, from an environmental perspective, the failures of energy policies in most countries hinder further developments in recycling of materials with energy values. LCA is still considered to be the best method to quantify and therefore compare the impact on the environment. Consequential LCA is widely considered as a suitable policy support because of its ability to model consequences to the greatest extent. But whether LCA can serve as a policy tool is the question of circular economy and its role as a policy support the question arises whether or not the consequences we model lead to constructive results. In case the consequence for a region is to quit recycling materials because of an (environmentally) inappropriate energy mix, a development of recycling technologies will be stopped that could actually be benefited in the future. It is not the recycling that is not environmentally favourable, it is the energy strategy hence the energy mix of a country. Yet recycling is blamed for not being the best environmental option. This means that we chose options that will lead to disadvantages in the future. Even though LCA grasps the environmental aspects quite well, the problem clearly lies in asking the right questions rather than mixing up different aspects of the same problem. Even if the LCA results are clear at first sight, it is not always clear in the future. It is not the recycling that is not environmentally favourable, it is the energy strategy that is not environmentally favourable.

178 Multicriteria optimization of a biofinery for the production of bio based flame retardant and other bio products

O. Mrani, TECHNISCHE UNIVERSITÄT DÄRFSTADT / BAUNG. L. Schebek, Technische Universitaet Darmstadt / Material Flow Management and Resource Economy

The project PHOENIX, which is funded by the European Union (EU), aims at the development of non-halogenated flame retardants based on biogenic resources. The research investigation covers the entire spectrum from developing materials to their industrial application. As part of the project, LCA is carried out for decision support to all stakeholders involved in the development process. In order to deliver support strategies for decision processes, LCA has to take into account that a biofinery system is composed from several production lines, multiple biomass resources and multiproducts. To tackle this complexity, optimization methods can be used in order to speed the analysis of the multitude of variant under consideration during technology development. We selected the optimization method analytic hierarchy process (AHP) knowing that AHP has, not only, been frequently applied in MCDM, but it also provides userfriendly support to decision making group by simplicification and decentralization of complex decision making. A biofinery is divided to the mainly divided into three levels, which in turn are divided into subcategories. The first top level includes the entire biofinery (top management level, management of the entire biofinery and overall chemical production-platform). The second level represents the production lines, which correspond to the desired end products of the biofinery (management of the production line, for example production of biomass-based panel etc.). These production lines are generated nonconsecutive process chains which are on the third level (line of individual process for example cultivation of biomass, transport etc. Each process chain again contains several alternatives with multiple indicators which are optimized as to finding and designing the final biofinery. In the proposed biofinery framework, the weighting of the criteria and interests changes depending on the hierarchical level. Thus means that one criterion may be relevant as to one specific level and at the same time insignificant for the other levels. The optimization result factors in the interests of all three levels (overall management, production management and processing).
Environmic multi-objective optimization of an eco-industrial park
L. Lessard, Quantis; P. Collet, IDEEL; M. Kermani, F. Maréchal, École Polytechnique Fédérale de Lausanne

A circular economy refers to an industrial economy with a cradle to cradle vision, shifting from the use of fossil fuels towards renewable energy, minimizing the use of toxic chemicals and reducing waste by recycling through careful system design. The concept of a circular economy is based on the idea that nothing is lost, in particular living systems, with a major outcome being the optimization of systems rather than of the individual components which make up each system. Industrial symbiosis (IS) is the exchange of energy, waste and by-products among industries in order to add value, while reducing costs and environmental impacts. This is consistent with the principles of a circular economy, and is a stepping stone towards the design of sustainable economies and policies. In the scope of this study, the use of local and renewable resources and the development of industrial ecology can contribute to the decrease of environmental impacts of a living, interactive system, on a given territory. A process system design framework is developed to model a superstructure that contains the list of possible options for the system. The LCA is used to calculate the environmental impacts of a product or a service. The method requires certain adjustments in order to assess the impacts of a spatially delimited system. The design of such system can be done by combining Life Cycle Assessment (LCA) that can provide a clear and structured framework to assess the environmental impacts of the system and process system design methods that applies mathematical programming techniques to systematically design territorial configurations using costs and thermo-chemical or physical models. The new method allows for the use of a systematic modeling framework and using an optimisation approach that allows to generate a limited set of pareto optimal configurations that can then be evaluated to identify the most attractive solution. The method has been successfully applied on a case study of a city with 100,000 inhabitants.

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Environmental benefits of industrial symbiosis for the steel industry: Application of stainless steel slag
A. Di Maria, KU Leuven / MTM; M. Salman, M. Dubois, K. Van Acker, KU Leuven

The steel industry is a key sector in the implementation of the Industrial Symbiosis (IS). Despite a wide literature on strategies for IS in steel industry, research should focus on the optimisation of those residues whose potential for recycling is not explored at present. The current study presents an environmental evaluation of a novel process aiming at the recycling of Stainless Steel Slag (SSS) as a binder for the production of construction materials. Currently, SSS is stabilized with borates added in a dry phase and recycled as low quality aggregate due to the chromium content. Moreover the dusty texture makes the handling difficult. Therefore research for more sustainable solutions is needed. Thermo Alkali-Activation (AA) process can be applied to SSS to produce a new binder for construction materials. The process activates the binding property of SSS through the addition of alkali silicate in a high temperature environment, hence foregoing the need of stabilization by borates. For the presented study, two different blocks (S-Blocks) were developed through AA using SSS slag as binder: 1) Solid S-Block- similar to traditional paver concrete block and 2) Aerated S-Block- with a porous internal structure similar to traditional aerated block. A Life Cycle Assessment (LCA) using a “cradle to gate” approach has been conducted in order to assess the environmental impacts of three different scenarios: Scenario 1 (no valorisation)- SSS is stabilized through borates addition and sold in the market as low-quality aggregate, Scenario 2 (Solid S-Blocks valorisation)- SSS is valorised through production of 1m² of Solid S-Blocks and Scenario 3 (Aerated S-Blocks valorisation)- SSS is valorised through production of 1m² Aerated S-Blocks. Scenarios 2 and 3 have been further compared with LCA on production of 1m² traditional blocks with similar properties that are already available in the markets. The LCA results show that the two valorisation scenarios have similar (scenario 2) or higher (scenario 3) impacts compared with scenario 1. However, the production of S-Blocks has lower impacts compared to production of their substitutes in the market. The environmental analysis indicates the advantages of industrial symbiosis in stainless steel production industry. LCA acted as a powerful tool to assess the environmental performances of new waste valorisation practices and the impacts of the application of IS.

Ecological Risk Assessment and Sustainable Management of Contaminated Sediment: Perspectives and Experiences

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A selection of sediment quality benchmarks for ecological risk assessment: contamination of DDT and Hg in Lake Maggiore (Italy)
L. Marziali, IRSA-CNRR (Brugherio); L. Guzzella, Italian National Research Council Water Research Institute CNRIRSA; D. Rabuffetti, Regional Agency for the Protection of the Environment of Piedmont / Department of Verbano Cusio Ossola

Lake Maggiore in Northern Italy is characterized by residual DDT and mercury contamination of sediments caused by the activities of a factory located nearby of the Toce River, one of the main tributaries of the lake. An environmental risk assessment (ERA) analysis is necessary to assess the potential risk for the aquatic ecosystem. For what concerns exposure, contaminated sediments were proved to be the main exposure pathway for the aquatic fauna. Therefore one of the focal point of the ERA was the study of the site-specific bioavailability of DDT and Hg. Bioavailability was assessed using different approaches: 1) analysing bioaccumulation in benthic invertebrates belonging to different taxonomic functional groups deploying passive samplers like PE (Polyethylene membrane) loaded with PRC (Performance Reference Compounds, i.e. labelled-DDs compounds), and Diffusive Gradients in Thin Films Spheron-Thiol-agarose passive samplers (DGT). For what concerns sediment quality benchmarks, the selection of proper thresholds was challenging, being intended to predict different levels of risk associated with sediment toxicity. At first, a comparison with national sediments was performed in order to identify screening levels considered safe (i.e. no effect) for the ecosystem. In case of higher contaminant concentrations, as in the case of the Toce River and the Pallanza Bay in the lake, further benchmarks (e.g. probable risk, moderate level effect, severe level effect) were selected. At this purpose, a review of standard toxicity tests and SSD calculations for benthic invertebrates from the scientific literature and from existing toxicity databases was carried out. Similarly, published papers reporting body burdens and toxic effects for invertebrates were analysed. Values of acute and chronic NOEC, LOEC, LC50, EC50, HC50, HC95, were selected for defining different risk levels. The selection of proper benchmarks for the ecological risk assessment poses strong implications for environmental management, leading decision for remediation measures.

182 Bioavailability and toxicity of sediment-bound contaminants (PCBs and metals) using an integrative approach: a case study in the Venoge river (Switzerland).
L. Guzzella, KU Leuven; E. Ewag, Aquatic Research; M. Casado-Martinez, Centre Ecotox; N. Esteppepy, University of Lausanne; F. de Alencastro, EPFL Swiss Federal Institute of Technology in Lausanne; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology; B. Ferrari, Ecotox Centre CH

The Venoge is a small river flowing into Lake Geneva, the largest water body of Switzerland. Previous monitoring campaigns have shown increased concentrations of PCBs in fishes, which were reported to exceed maximum levels set by the European Union for human consumption. Water quality of the river is well characterized, both in terms of chemical contamination and benthic macroinvertebrate community structure. However, no information of sediment quality is available so far. To assess the potential impact of sediment bound contaminants on the aquatic ecosystem, an integrative methodology was applied that includes sediment physico-chemical measurements, a battery of sediment toxicity tests, and measurement of contaminants in benthic invertebrates. Sediments were collected at several sampling sites along the Venoge and analyzed for PCBs and metals as well as ancillary parameters important for determining contaminant bioavailability. The battery of tests used for the characterization of sediment ecotoxicity included the growth inhibition test using the aquatic macrophyte Myriophyllum aquaticum (ISO 16191); the growth and mortality test using the ostracod Heterocylops incongruens (ISO 14371); and the emergence test using midge larvae (Chironomus riparius, AFNOR 90-339). Bioaccumulated contaminants were assessed in midge larvae. Four sampling sites were selected following the identified sources of contamination and previous results of water quality. Results of the toxicity tests showed no effect on ostracod mortality but growth inhibition at the sampling site closest to the mouth of the river. On the contrary, percent emergence of midge larvae was decreased only at the site closest to the effluent outfall of the waste water treatment plant. Chemical analyses of sediments and midge larvae are on-going. The results will be discussed based on the two objectives of this study: 1) to assess whether sediments are contributing to the impairment of the beneficial uses of this aquatic ecosystem (ecosystem services) and 2) to assess which of the selected tools can provide useful information within a decision-making framework.

183 Regional Sediment Quality Guidelines within the European Water Framework Directive: strengths, weaknesses, opportunities and threats. The Rhine case as case study
I. Menchaca, Marine Environment Department; J. Rodríguez, Boston, J. Franco, Azti-Tecnalia; J. Armendia, AZTI Foundation; J. Larretxe, M. Belzunce, Azti-Tecnalia

The regional SQG values developed in the present study, using different approaches and basic biological level approaches, were intended to be used as guidance values for project managers and decision makers, in assessing chemical and physicochemical status in marine waters, to be used within the European Water Framework Directive. The aims of this study are (i) to calculate the regional SQG for metals and organic compounds (PCBs and PAHs) for the Basque coast (Bay of Biscay), based upon the chemical, toxicity and benthic biotic index values, (ii) to compare these regional SQG values with international values and, (iii) to carry out SWOT analysis

184 Contaminated sediments in backwaters of the Elbe - do they pose a risk to the river’s quality?
N. Esteppepy, University of Lausanne; F. de Alencastro, EPFL Swiss Federal Institute of Technology in Lausanne; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology; B. Ferrari, Ecotox Centre CH
S. Heise, Hamburg University of Applied Sciences / Life Sciences; U. Rieth, Institut für Hygiene und Umwelt

The German Elbe river flood plain has more than 1000 individual backwaters covering an area of altogether 50 km². In the context of setting up a concept for a basin-wide sediment management by the International Commission for the Protection of the Elbe (IKSE), this study was initiated in order to inform the IKSE on the relevance of backwater sediments for the quality of the Elbe. At the same time, it was crucial to examine the contamination of those sediments and to what extent they were exchanged with the major river. In 2013 and 2014, 25 backwaters were investigated with regard to sediment contamination, ecotoxicology, erosion stability, and thickness of the potentially resuspendable sediment layer. Dating of selected cores was done by measuring activity of 137Cs and by determination of heavy metal ratios. Management measures may become necessary, but need to be based on not only exceedance of EQS but also on ecotoxicological responses and a profound system understanding.

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Do International Dredged Material Frameworks Effectively Assess Risk? S.E. Apitz, SEA Environmental Decisions Ltd; S. Agius, Environment Canada

The objectives of many countries’ Disposal at Sea (DaS) Programs, and of related national and international legislation, mirror the 1996 Protocol to the London Convention (London Protocol) objective “to protect and preserve the marine environment from all sources of pollution and take effective measures, according to the scientific, technical, and economic capabilities, to prevent, reduce and where possible eliminate pollution caused by dumping or incineration at sea of wastes or other matter.” The development of DaS assessment frameworks for dredged material can involve almost endless possible permutations of action lists, benchmarks, and decision rules. Both individual choices about these parameters, and how they are combined can affect potential regulatory outcomes and the efficacy of task or decision. Management measures may become necessary, but need to be based on not only exceedance of EQS but also on ecotoxicological responses and a profound system understanding.

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Guidance and risk assessment schemes for prospective sediment toxicity testing of organic chemicals N. Diepen, Wageningen University / Department of Aquatic Ecology and Water Quality Management; b. hauveco, Environmental Risk Assessment Team; P.J. van den Brink, Alterra and Wageningen University; M. Van den Heuvel-Greve, Wagningen IMARES / Marine Coastal Systems; A.A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group; T.C. Brock, Alterra, Wageningen University and Research Centre / Environmental Risk Assessment Team

A framework for prospective risk assessment of sediment-bound organic chemicals is currently lacking. Such a framework requires clearly defined protection goals, evidence-based concepts that link exposure to effects, and a transparent tiered effect assessment. Here, we discuss possible specific protection goals based on the ecosystem services concept for future prospective sediment RA procedures for organic chemicals in sediment. Benthic organisms provide important ecosystem functions and services, which are similar between freshwater and marine ecosystems. Key drivers for sediment-related ecosystem services are microorganisms, benthic algae, sediment-rooted macrophytes, benthic invertebrates, and benthic vertebrates. Triggers for sediment testing based on chemical properties and toxicity are required. Sediment-spiked laboratory toxicity tests are the standard test species for endpoints of teratogenicity and sublethal endpoints. The range of taxonomic groups for sediment testing needs to be extended. Prior to actual testing a cost-effective screening based on aquatic toxicity data and equilibrium partitioning is advisable. Assessment factors to be used in tier-1 effect assessment as well as the feasibility of the SSD approach as a tier-2 option are discussed. Micro- and mesocosm experiments with spiked sediment are proposed as a 3rd experimental tier. An outlook to unifying information from the various tiers by experimental work, fate modelling, and effect modelling as cost-effective prognostic tools for sediment RA is provided.

Engineering in vivo fish models for advancing environmental hazard and risk assessment

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Introduction to the session events and our focus on transgenic models in ecotoxicology C.R. Tyler, Biosciences College of Life and Environmental Sciences; A. Takeonso, J. Moreman, University of Exeter; J. Green, University of Exeter / College of Life and Environmental Sciences; R. Cooper, University of Exeter; A. Foreman; S. Mourabii, A.R. brown, T. Kudoh, University of Exeter

Transgenic fish are applied in ecotoxicology where they have the potential to provide integrated systems for assessing health impacts of chemicals. Zebrafish and medaka are the most popular fish for the generation of transgenic models, due to their high fecundity, transparency of their embryos (zebrafish), rapid organogenesis, and availability of extensive genetic resources. Transgenic fish models are now available to study for the effects of heavy metal toxicity (via heat-shock protein genes), oxidative stress (via an electrophile responsive element), various organic chemicals acting through the aryl hydrocarbon receptor, thyroid and glucocorticoid response pathways, and estrogenicity, using a spectrum of different fluorescent markers. Due to variations in their sensitivity, only a few transgenic models are capable of detecting responses at environmentally relevant exposures. Even so, they provide valuable systems to study the biological mechanisms of chemical toxicity. A favoured method for the study of gene expression in transgenic fish is the two step activation Gal4-UAS system. In this system, GAL4 is placed under the control of a desired promoter and UAS is placed next to a desired reporter gene, such as a fluorescent marker; cells producing both components of the system will activate the reporter gene. Transgenic fish systems provide powerful tools for the study of chemical effects, especially in mutant lines lacking skin pigmentation where responses can be visualized in real time and across multiple target tissues in intact organisms. In this presentation we will introduce the topic of transgenic fish for studies in environmental toxicology and provide a viewpoint on future development opportunities, and constraints, for their use in risk assessment.

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What constitutes a model organism in ecotoxicology? H. Segner, University of Bern / Centre for Fish and Wildlife Health

Transgenic fish are applied in ecotoxicology where they have the potential to provide integrated systems for assessing health impacts of chemicals. Zebrafish and medaka are the most popular fish for the generation of transgenic models, due to their high fecundity, transparency of their embryos (zebrafish), rapid organogenesis, and availability of extensive genetic resources. Transgenic fish models are now available to study for the effects of heavy metal toxicity (via heat-shock protein genes), oxidative stress (via an electrophile responsive element), various organic chemicals acting through the aryl hydrocarbon receptor, thyroid and glucocorticoid response pathways, and estrogenicity, using a spectrum of different fluorescent markers. Due to variations in their sensitivity, only a few transgenic models are capable of detecting responses at environmentally relevant exposures. Even so, they provide valuable systems to study the biological mechanisms of chemical toxicity. A favoured method for the study of gene expression in transgenic fish is the two step activation Gal4-UAS system. In this system, GAL4 is placed under the control of a desired promoter and UAS is placed next to a desired reporter gene, such as a fluorescent marker; cells producing both components of the system will activate the reporter gene. Transgenic fish systems provide powerful tools for the study of chemical effects, especially in mutant lines lacking skin pigmentation where responses can be visualized in real time and across multiple target tissues in intact organisms. In this presentation we will introduce the topic of transgenic fish for studies in environmental toxicology and provide a viewpoint on future development opportunities, and constraints, for their use in risk assessment.

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Rapid fluorescent detection of (anti-)androgens with spg1-gfp medaka A. Sibillito; P. Damdimopoulou; Y. Ogino; P. Spighanzlova, Watchfrog S.A.; S. Miyagawa, Natural Institute of Natural Sciences; D. Du Pasquier, Watchfrog S.A.; N. Mouatassim; T. Iguchi, National Institute for Basic Biology / Molecular Environmental Endocrinology; G.F. Lemkine, Watchfrog S.A.; B.A. Demeneix; A.J. Tindall, Watchfrog S.A.

Environmental anti-androgen contamination is widespread and has been associated with negative impacts on biodiversity and human health. Our aim was to develop a transgenic medaka model bearing a sensitive and specific androgen responsive fluorescent reporter construct for whole organism-based environmental screening for pro- and anti-androgens. Putative spg1 promoters were identified within the genome of the stickleback (Gasterosteus aculeatus) and cloned upstream of GFP. A number of putative androgen and estrogen response elements were identified in each promoter. Gemineral transgenesis with spg1-gfp led to stable medaka lines. GFP induction was restricted to the kidney, the site of spg1 protein production in sticklebacks. Significant dose-dependent gfp expression was induced by androgen treatment of newly hatched fry, but not by estrogens, mineralocorticoids, glucocorticoids or progestogens. In addition to flutamide, the biocides fenitrothion, vinclozolin and linuron significantly inhibited 17MT-induced GFP induction, validating the model for detection of anti-androgens. The spg1-gfp medaka model provides a sensitive, specific and physiologically pertinent biosensor system for analyzing (anti-)androgenicity of raw chemicals or environmental samples.
Application of ERE-TG zebrafish in assessing health impacts of oestrogenic EDCs on embryo development and sex differentiation

T. Kudoh, University of Exeter / Biosciences College of Life and Environmental Sciences; A. Takesono , University of Exeter; O. Lee, University of Exeter / Biosciences College of Life and Environmental Sciences; O. Kah, IRSET; C.R. Tyler, Biosciences College of Life and Environmental Sciences

We have developed an in vivo method with transgenic zebrafish (ERE-GFP TG fish) that detects tissue responses to oestrogenic chemicals via GFP expression in vivo. Using this TG fish, we have observed that a variety of tissues respond to oestrogens including the liver, heart, muscle and forebrain. However, the roles of oestrogen signaling in development and functions of these tissues are largely elusive. We found that cells in telencephalon and olfactory bulb express GFP in response to 17-α-ethynyl estradiol (EE2) from early stages of neurogenesis and the number of GFP cells subsequently increases in larvae up until they are 2 weeks old. The oestrogen responsive telencephalic neural cells (TC-GFP+) cells show characteristic polar morphology with radial process, suggesting they may be radial glia cells, well-known neural stem cells. TC-GFP+ cells do not possess immunoreactivity for neuronal markers (acetylated tubulin and HuC), but express a neural stem cell marker Sox2. These data suggest specific roles of oestrogen signaling in neural stem cell development and functions. To further analyse behaviour of oestrogen responding brain cells in normal and polluted environment, we are currently modifying our ERE-GFP TG fish to create different transgenic fish lines. Firstly, we have generated a double transgenic line which responds to oestrogen and expresses two fluoroscence colours derived from two oestrogen responsive promoters that can amplify ERE response with mCherry promoter and epl9a promoter with GFP reporter. Heterozygous [ERE-mCherry] x [Cypl9a-GFP] embryos show that some cells in olfactory bulb respond to both transgenes upon EE2 exposure, but there are fewer cells of mCherry and GFP double positive cells observed in the posterior part of telencephalon. We are also creating ERE-Kaloo and ERE-GCAMP TG fish lines. Kaloo line would permanently label the oestrogen responding cells, facilitating cell lineage tracing in juvenile to adult stage. Calcium activatable GCAMP fluorescent proteins visualise neuronal activity in the oestrogen responding cells. These models will now be applied to help better understand the functional roles of oestrogens in development of the brain and sex differentiation in fish. Findings to date imply heterogenic roles of oestrogen signalling during brain development.

191 Zebrafish reporters of glucocorticoid and aryl hydrocarbon receptor activity

D. Gorelick, J.L. King, B.L. Chine, University of Alabama at Birmingham Exposure to environmental endocrine disruptors is a significant health concern. Previous work has focused on detecting estrogens, however other chemical classes, such as glucocorticoids and dioxins, are also relevant contaminants. Here we describe the development of new zebrafish reporters for the detection of glucocorticoid receptor (GR) or aryl hydrocarbon receptor (AhR) activity. Previous zebrafish reporters were limited by low sensitivity or progressive silencing of reporter gene expression. To overcome these limitations, we employed a bipartite responsive promoter that can amplify ERE response with mCherry reporter and epl9a promoter with GFP reporter. Heterozygous [ERE-mCherry] x [Cypl9a-GFP] embryos show that some cells in olfactory bulb respond to both transgenes upon EE2 exposure, but there are fewer cells of mCherry and GFP double positive cells observed in the posterior part of telencephalon. We are also creating ERE-Kaloo and ERE-GCAMP TG fish lines. Kaloo line would permanently label the oestrogen responding cells, facilitating cell lineage tracing in juvenile to adult stage. Calcium activatable GCAMP fluorescent proteins visualise neuronal activity in the oestrogen responding cells. These models will now be applied to help better understand the functional roles of oestrogens in development of the brain and sex differentiation in fish.

192 Development and validation of an OECD test guideline incorporating a transgenic fish model

F. Brion, INERIS / Ecotoxicology Unit; B. PICCINI, C. TURIES, INERIS; J. Porcher, INERIS / UMRI SEBIO; J. Green, University of Exeter / College of Life and Environmental Sciences; A. TAKESONO, University of Exeter; C.R. Tyler, Biosciences College of Life and Environmental Sciences; E. Fetter, Department of Bioanalytical Ecotoxicology; S. Scholz, Helmholtz Centre for Environmental Research / Department of Bioanalytical Ecotoxicology; J. Nicolau, O. Kah, IRSET

Concern about the effects of Endocrine Disrupting Chemicals (EDCs) to fish and humans reproductive health has stimulated the development of tests to identify endocrine active substances and quantify their effects. Despite important progress made in that direction during the past decade, one major challenge to ecotoxicology is still to develop specific biological tools that allow rapid and cost-efficient assessment of EDCs for environmental hazard and risk assessment. In 2012, an in vivo mechanism-based zebrafish embryo assay was developed (Brion et al., 2012) and called EASZY for Detection of Endocrine Active Substances, acting through estrogen receptors, using transgenic cypl9a1a1b-GFP Zebrafish embryos. The relevance of the EASZY assay for assessing the estrogenic potency of test substances was evaluated by testing more than 60 chemicals and it was found that, in the EASZY assay, GFP was induced in a ER-specific manner by (i) compounds that bind directly to estrogen receptors as agonists (ii) compounds that require metabolic activation into estrogenic metabolites (iii) aromatizable androgens as well as some non aromatizable androgens. Since the EASZY assay emerged as a fast, simple, specific and sensitive in vivo assay for quantifying the estrogenic potency of chemicals at very early critical developmental stages, a Standard Project Submission Form (SPSF) was submitted by the lead country France to OECD test guidelines program in 2012 and the EASZY was accepted to the Validation Management Group for Ecotoxicity (VMG-Eco) working program in December 2013. The first objectives of the validation were (i) to ensure training persons to conduct the test (ii) to assess the transferability of the test and the test method to the participating laboratories (iii) to assess the intra- and inter-laboratory reproducibility by testing a set of reference ligands including both positive and negative test substances. At the occasion of this oral presentation the current progress of the validation of the EASZY assay will be presented while considering the strengths (and the limits) of the transgenic models for environmental hazard and risk assessment. Acknowledgement – Study support by the DRC-50 “Perturbateurs Endocriniens et impacts environnementaux” of the French Ministry of Environment.

193 Application of a transparent transgenic zebrafish model for chemical testing in a semi-automated image analysis system

J. Green, J. Metz, A.R. brown, S. Owen, T. Kudoh, University of Exeter; C.R. Tyler, Biosciences College of Life and Environmental Sciences

Zebrafish have emerged as a powerful tool for use in in vivo chemical and drug screening because of attributes including their rapid development, relative transparency, high fecundity, and genetic and morphological similarity to mammals. With fluorescence protein expression linked to specific enzymes or receptors, transgenic zebrafish models offer systems for identifying and quantifying molecular initiating events (MIEs) that may trigger adverse outcome pathways (AOPs). With new automated imaging and image analysis systems being specifically designed or adapted for zebrafish larvae (Pardo-Martin et al. 2010, Romano and Gorelick 2014), the application of transgenic zebrafish models for medium-throughput chemical screening is an emerging prospect. We present a novel transgenic zebrafish model for application in a semi-automated imaging and image analysis system for screening of estrogenic compounds. This system provides quantitative and qualitative analysis of tissue-specific targeting of estrogenic chemicals. This is visualised via a GFP fluorescence response in a well-established transgenic model (Lee et al. 2012) that has been incorporated into a non-pigmented ‘Casper’ phenotype. The Casper phenotype allows for easier tissue identification and reduces issues relating to body orientation and fluorescence signal blocking when imaging. With the increasing need to identify endocrine disrupting chemicals and characterise their effect on tissues and organs in humans and other animals, the Casper estrogen responsive GFP transgenic zebrafish in a semi-automated system offers an excellent tool for screening chemicals for estrogenic activity in whole organisms. References: Pardo-Martin C, Chang TY, Koo BK, Gilleland CL, Wasserman SC, Yanik MF (2010) Nat Methods. 7(8):634-6. Romano SN, Gorelick DA (2014) JoVE. 17;(87) Lee O, Takesono A, Tada M, Tyler CR, Kudoh T (2012). Environ Health Perspect. 120(7):990-996.

194 Transgenic fluorescent tadpoles for detecting chemical disruption of the thyroid axis

P. Spiranuizlova, Watchfrog; A.J. Tindall, G.F. Lemkine, D. Du Pasquier, Watchfrog S.A.

During the last decades, there has been increasing concern about thyroid axis disrupting contaminants present in the environment. As the correct functioning of the thyroid axis is crucial for vertebrate development, it is necessary to develop a rapid method to detect thyroid axis disrupting chemicals. One of the most studied hormone-regulated developmental processes in vertebrates is anuran amphibian metamorphosis, a process associated with great morphological and physiological changes fully dependent on thyroid hormones. Leucine zipper transcription factor (TH/bZIP) is highly up-regulated during metamorphosis of Xenopus laevis and can be induced prematurely by thyroid hormones. We developed a transcriptional-based assay for the detection of thyroid axis disruption, which uses transgenic Xenopus laevis tadpoles harbouring gfp coding sequence under the control of the TH/bZIP promoter. Our test is a rapid and simple solution for screening large number of molecules or testing environmental samples.

195 Final Discussion

C.R. Tyler, Biosciences College of Life and Environmental Sciences

Ecotoxicology of amphibians and reptiles. Resolving uncertainties about the impact of pollutants on individuals, populations and communities

C.R. Tyler, Biosciences College of Life and Environmental Sciences

Tolerance inheritance to copper in the Perce’s frog.

T. Fasola, CESAM Dept of Biology; R. Ribeiro, University of Coimbra; I. Lopes,
University of Aveiro / CESAM Biology Department
Chemical contamination may lead to the occurrence of genetic erosion in natural populations. This is of particular concern if the loss of genetic variability is irreversible due, for example, to the contaminant-driven elimination of alleles when chemical tolerance is a fully or incomplete recessive (or incompletely dominant) trait, or to the occurrence of recessive tolerance inheritance. To counter in natural populations of the frog species *Pelophylax perezi*. Twenty egg masses, in Gosner stage 8-10, were collected from a population inhabiting a metal free pond and exposed to copper sulphate plus to a control (FETAX medium). Time to death was registered for each egg, with observations following a logarithmic time scale: 120min, 160min, 235min, 320min, 470min, 670min, and 950min. For each egg mass, the median lethal time (LT$_{50}$) and respective quartiles (LT$_{25}$ and LT$_{75}$) were computed. Subsequently, critical sensitive egg masses (categorized as those with an LT$_{50}$ similar or below the average of the set of LT$_{50}$ for all egg masses) were identified. The within egg mass variability in time to death responses was evaluated through its relative spread: the difference between the lower and upper quartiles relatively to the median: [LT($50$)−LT($25$)]/LT$_{50}$. If metal tolerance corresponded to a recessive trait then the most tolerant egg masses (both parents being recessive homozygotes) would present relative spreads lower than at least some of those with an intermediate LT$_{50}$ (both parents being heterozygotes), which was not the case: no relationship between relative spreads and LT$_{50}$ values could be found. Therefore, the recessive tolerance inheritance hypothesis was not supported by the presented cell. On the other hand, when spermatozoa were polarized *Xenopus laevis* full range of variability in the observed pattern of death per observation. This fact suggests that the possible genetic mechanism of tolerance could be determined by co-dominance.

197 Xenopus laevis oocyte maturation is affected by metal chlorides S. Slaby, University of Lille / Lille Sciences et Technologies Equipe Régulation des Signaux de Division UGSF UMR Cité scientifique SN Villeneuve d’Ascq France; S. Lemiére, Fonctionnement des Ecosystèmes Anthropisés; G. Marchand, PRES Univ Lille Nord de France / Univ Lille1; S. Demuyck, LGCE - EA 4515; A. Gelade, J. Bordart, University of Lille / Lille Sciences et Technologies Equipe Régulation des Signaux de Division UGSF UMR Cité scientifique SN Villeneuve d’Ascq France; M, Marin, University of Lille / Biology Few studies have been conducted using Xenopus laevis germ cells as oocytes, though these cells offer many advantages allowing both electrophysiological studies and morphological examination. The aim of this work was to investigate the effects of metal and cobalt exposures using germ cell biology approaches. First, cell survival was evaluated with both phenotypical and electrophysiological approaches. Secondly, the effect of metals on oocyte maturation was assessed by morphological observations (germinal vesicle breakdown: GVBD) and by electrophysiological recordings (calcium-activated chloride currents). From survival experiments, our results showed that metal chlorides induced a dose-dependent decrease in cell viability. Chloride currents were recorded in oocytes as they develop. In Xenopus laevis oocyte, the presence of chloride in the media is essential for the development of the oocyte. The results obtained with metal chlorides demonstrated that Xenopus laevis oocyte provide an interesting lab model to evaluate the toxicity of aquatic contaminants.

198 Risks of progesterogens to amphibians - Low ng/L concentrations disrupt egg development M. Saltholm, Uppsala University / Dept of Environmental Toxicology; J. Fick, Umea University / Department of Chemistry; C. Berg, Dept. of Environmental Toxicology / Dept of Environmental Toxicology Several progesterogen pharmaceuticals, including levonorgestrel (LNG), norethindrone (NET), and progesterone (P4), are present in the aquatic environment at low ng/L concentrations. The aim of the present study was to compare the sensitivity of male and female gametogenesis (spermatogenesis and oogenesis) in frogs to impacts of progesterogen exposure. Xenopus tropicalis males were exposed to LNG and females were exposed to LNG, P4, or NET at concentrations of 0–100 ng/L. Eggs were analyzed for abnormalities and changes in the mean frequencies of spermatozoa, spermatoctyes, and spermatids, the seminiferous tubule diameter, number of seminiferous tubules, number of germ cell nests, and amount of spermatozoa. Sperm count and sperm motility were determined in fresh testicular samples using a microscope counting chamber. Oocyte maturation was analyzed histologically. All three test substances caused reduced proportions of vitelligenic oocytes and increased proportions of previtellogenic oocytes compared with the controls, indicating inhibited vitellogenesis. These effects were ascertained at 1 ng/L for NET and LNG and at 10 ng/L for P4. No significant impact on the proportion of spermatozoa, spermatoctyes, or spermatids in the testes, or on sperm count or sperm motility was detected. The results imply that spermatogenesis and sperm quality are much less sensitive to progesterin toxicity than oogenesis in amphibians. Taken together our data show that vitellogenesis is a sensitive target for progesterogens and that female reproduction may be at risk in exposed wild frog populations.

199 Early life stage developmental effects of elevated maternal dietary selenium exposure in amphibians A. J. Masse, Toxicology Centre / Toxicology; D.M. Jain, University of Saskatchewan / Toxicology Centre; J.R. Muscatello, Stantec / Department of Toxicology Selenium (Se) is a contaminant of potential concern in aquatic systems located downstream of mining operations due to its efficient incorporation into food webs, potential for bioaccumulation at higher trophic levels, and role as a developmental toxicant in oviparous vertebrates. Adverse reproductive effects of Se have been the primary focus of research in fish and bird species; however studies focusing on Se toxicity in amphibians are lacking, particularly early life stage toxicities and tissue-based toxicity thresholds. The objective of this study was to determine dose-response relationships for early life stage toxicities in Xenopus laevis. Following a 68 day dietary exposure to food augmented with L-selenomethionine at measured concentrations of 0.7 (control), 10.9, 30.4, and 94.2 μg Se/g dry mass (d.m.), adult female X. laevis (n=9-10 per treatment) were bred with untreated males. Evaluations in the resulting eggs/embryos included Se concentrations, fertilization success, hatchability, survival, developmental rates, and the frequency and severity of Se-induced malformations. Percent hatchability of embryos and survival of embryos/tadpoles up to 5 days post-fertilization (dpf) were significantly reduced in the 30 μg Se/g treatment group compared to the control group (p<0.05). The measured Se concentration in subsamples of 100 eggs per female showed mean values of 1.6, 10.8, 28.1 and 81.7 μg Se/g d.m. for treatment groups receiving 0.7, 10.9, 30.4, or 94.2 μg Se/g d.m. in their diet, respectively. Analysis of 5 dpf tadpoles showed that embryos/tadpoles from the 81.7 μg Se/g d.m. group were affected with eye and gut, and lens structures were prevalent in addition to the occurrence of edema and eye degeneration. Both the frequency and severity of abnormalities were significantly increased in the 5 dpf tadpoles from the 81.7 μg Se/g embryo d.m. group when compared to the control (p<0.001). A strong positive relationship between the frequency of total abnormalities and egg selenium concentration was observed (r²=0.878, p<0.001). Future work will include an assessment of oxidative stress biomarkers in 5 dpf tadpoles to evaluate potential effects on antioxidant defense mechanisms and its relation to Se-induced malformations. Overall, this study aims to aid in further understanding the sensitivity of amphibians to Se, with the goal of developing environmentally relevant toxicity thresholds.

200 Understanding the population-level consequences of copper toxicity and climate variability on amphibians inhabiting contaminated environments S. M. Weig, Savannah River Ecology Laboratory / Savannah River Ecology Laboratory; C. J. Salice, Towson University / Biological Sciences Environmental Science; D. Scott, S.L. Lance, University of Georgia / Savannah River Ecology Laboratory A great deal of amphibian ecotoxicology data has been generated in the last 15 years, with excellent insights obtained from both laboratory and mesocosm studies. In wild populations, however, the relationship between individual-level contaminant effects and population persistence remains unclear; i.e., a single instance of exposure to lethal or sublethal concentrations will not necessarily lead to a population-level decline. A powerful tool to investigate the population-level consequences of contaminant toxicity is to incorporate the toxicity data into population models. Here we present a stochastic matrix population model for toads inhabiting a copper-contaminated environment. We parameterize the model with published demographic data available for the southern toad (*Anaxyrus terrestris*) and other *Anaxyrus* species when southern toad data were not available. We also use data from a long-term study on the Savannah River Site (SRS) to provide a range of values for parameters for which no previous toad data are available. We used laboratory-generated toxicity data to inform adjustments to survival parameters for embryos and larvae in the model. We investigated a range of copper concentrations that correspond to laboratory data and the full range of survival (0-100% survival) for embryos and larvae. We also incorporated catastrophic reproductive failure which has previously been shown to be important for amphibian population persistence. Data collected on the SRS suggests that the probability of catastrophic reproductive failure is increasing in recent years. We incorporate stochasticity into all parameters in the model, except larval survival which we determined by a density dependent function. Our results suggest that copper toxicity in the embryo and larval stages is compensated by density dependence, so has little effect until complete mortality (i.e., 100% larval mortality). Catastrophic reproductive failure is a more important parameter in terms of affecting population persistence, which has been reported previously and is quickly coming to consensus. Future research efforts include modeling additional species (and associated copper toxicity) while also adopting a metapopulation approach to model a landscape of wetlands that vary in failure probabilities.

201 Occurrence and accumulation of contaminants in amphibians in diverse
landsca pes across the United States
Contaminants, acting singly or in combination with other stressors, are one of several potential causes of amphibian population declines. Surveys of amphibian populations have shown correlations between declines and proximity to both agricultural and urban lands. Moreover, contaminants are considered important co-factors that can disrupt both system responses, potentially increasing disease prevalence in local populations. The US Geological Survey has been conducting field studies throughout the United States designed to address the occurrence of contaminants in amphibian habitats and their accumulation in tissues to begin to understand the potential impacts on populations. Novel screening techniques are being utilized to assess the biological activity and inorganic composition of amphibian habitats in the Northeast to help prioritize areas of interest biologically and chemically for more in depth studies. As part of these studies, habitat quality (water and sediment) and amphibian health (accumulation into tissues) are being assessed in a variety of landscapes including remote high elevation locations in the Sierra Nevada Mountains, agriculturally dominated wetlands in Iowa, and National Wildlife Refuges and National Parks in the Northeast. In agricultural areas, the occurrence of pesticides in habitat and the accumulation in tissue was significantly correlated with land-use within a 5 km buffer of the wetlands. However, in remote and/or protected areas habitat quality and tissue body burdens was not correlated with the surrounding land-use because atmospheric transport is considered the dominant source of contaminants to these areas. From an ecosystem health standpoint, results suggest that amphibians are accumulating a wide variety of contaminants including several pesticides not previously reported in tissue. Although the types and concentration of contaminants detected in tissues varied by study area, the information gained will help prioritize particular compounds of interest for future studies. Amphibians residing and breeding in many of these diverse landscapes are exposed to contaminants throughout their life cycle and the potential effects of such exposures are currently unknown. Future research is needed to understand the interaction between contaminants and other stressors such as disease, at the population level.

Interpreting Biological Effects of Metals and Their Mixtures (I)

202 Metal mixture complexity: The antagonistic action of a Ni-Zn-Cu mixture to C. dubia becomes additive when Cd is added
C. Nys, K.L. Smalling, C. van Dijk, J.A. van den Berg, University Ghent / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; C.J. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research Department of Biology; E.E. Smolders, Katholieke Universiteit Leuven / Division Soil and Water Management; K.A. De Schamphelaere, Ghent University (UGent) / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit
There is a need for more understanding about combined and interactive effects of metal mixtures during chronic exposure to be able to incorporate metal mixture toxicity effects into risk assessment procedures. Therefore, we investigated the possible interactive effects in ternary and quaternary mixture combinations of metals, Ni-Zn-Cu and Ni-Zn-Cu-Cd, respectively on the reproductive toxicity of Ceriodaphnia dubia using a raya design in a modified natural water (1‘Ourthe, Oriental, Belgium: pH=7.3, Dissolved organic carbon= 7.3 mg/L and hardness=84 mg CaCO₃/L). Furthermore, we also investigated the influence of varying the metal concentration ratios within the ternary mixture on the magnitude of the interactive effects. Interactive effects were significantly antagonistic according to both the Independent Action (IA) and Concentration Addition (CA) model in the ternary Ni-Zn-Cu mixture. However, the type of interactive effect was dependent on the applied mixture concentration. At low mixture concentrations treatments, IA and CA predicted relative reproduction (RR) were not significantly different from observed RR, i.e., additive. When Ni, Zn and Cu were combined at higher mixture concentrations, the most significant antagonistic effects started to occur for both IA and CA. Varying the metal concentration ratios in the ternary mixture did not result in significant differences in the magnitude of the antagonistic effect. However, when Cd was added to the mixture combination, the global mixture effect became additive. The results of this study point out that there is a clear need to better mechanistically (e.g. physiologically) understand metal mixture interactions. Nonetheless, both CA and IA provide conservative models for predicting for C. dubia reproductive toxicity in Ni-Zn-Cu and Ni-Zn-Cu-Cd mixtures.

203 Interaction between nickel and cobalt toxicity in Enchytraeus crypticus is due to competitive uptake
E. Smalling, VU University Amsterdam / Department of Ecological Science; J. Baas, Centre for Ecology & Hydrology; C. van Gestel, VU University Amsterdam / Ecological Science
Uptake and toxicity of Ni-Co mixtures in Enchytraeus crypticus were determined after 4, 7, 10 and 14 days exposure in solutions embedded in an inert sand matrix. Generally, body concentrations of Ni and Co increased with increasing exposure concentrations. Ni body concentration was significantly reduced in the presence of Co, while Ni only marginally affected Co uptake. When expressed as free ion activities, individual toxicity of Ni and Co increased with time, with LC50s decreasing from 78.3 and 511 µM at 4 d to 40.4 and 393 µM at 14 d, respectively. When expressed as body concentrations, LC50₉₅ Ni,Co started to increase at lower concentrations. LC₅₀₉₅ Ni decreased during the first 7 d but remained stable afterwards. As identified by the MIXTOX model, interactions between Ni and Co were mainly antagonistic, when based on free ion activities, however, no interaction was observed when based on body concentrations. A process-based model, incorporating exposure time to analyse the mechanisms underlying the dynamic toxicity confirmed the differences in toxicokinetics of the two metals. Our findings suggest that body concentrations, which incorporate the bioaccumulation processes, are a better indicator of metal toxicity. The observed antagonism was mainly caused by competition between Co and Ni for binding sites and subsequent inhibition of Ni uptake. This competitive interaction occurred at the uptake level (toxicokinetics), but not at the target level (toxicodynamics).

204 Metal uptake and toxic growth effects in Lemma minor exposed to varying mixtures of uranium and cadmium
H. Lorens, K. Smalling, C. van Dijk, J.A. van den Berg, University Ghent / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; N. Nevejan, Ghent University / Laboratory of Aquaculture and Water Quality Management; A.R. de Schamphelaere, Ghent University (UGent) / Laboratory of Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; P. Kleeman, University of Antwerp / Systemic Physiological and Ecotoxicological Research Department of Biology; E.E. Smolders, Katholieke Universiteit Leuven / Division Soil and Water Management; K.A. De Schamphelaere, Ghent University (UGent) / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit
There is a need for more understanding about combined and interactive effects of metal mixtures during chronic exposure to be able to incorporate metal mixture toxicity effects into risk assessment procedures. Therefore, we investigated the possible interactive effects in ternary and quaternary mixture combinations of metals, U-Cd and U-Cd-Cu, respectively on the reproductive toxicity of Ceriodaphnia dubia using a raya design in a modified natural water (1‘Ourthe, Oriental, Belgium: pH=7.3, Dissolved organic carbon= 7.3 mg/L and hardness=84 mg CaCO₃/L). Furthermore, we also investigated the influence of varying the metal concentration ratios within the ternary mixture on the magnitude of the interactive effects. Interactive effects were significantly antagonistic according to both the Independent Action (IA) and Concentration Addition (CA) model in the ternary Ni-Zn-Cu mixture. However, the type of interactive effect was dependent on the applied mixture concentration. At low mixture concentrations treatments, IA and CA predicted relative reproduction (RR) were not significantly different from observed RR, i.e., additive. When Ni, Zn and Cu were combined at higher mixture concentrations, the most significant antagonistic effects started to occur for both IA and CA. Varying the metal concentration ratios in the ternary mixture did not result in significant differences in the magnitude of the antagonistic effect. However, when Cd was added to the mixture combination, the global mixture effect became additive. The results of this study point out that there is a clear need to better mechanistically (e.g. physiologically) understand metal mixture interactions. Nonetheless, both CA and IA provide conservative models for predicting for C. dubia reproductive toxicity in Ni-Zn-Cu and Ni-Zn-Cu-Cd mixtures.
well, based on the concentration addition model although some antagonistic effects are observed at low Cu concentrations. This is not the case for the Cu/Ni mixture. On the one hand, there is a strong synergistic effect on Cu toxicity when the larvae are exposed to Ni concentrations as low as 2-5% of the Ni EC50 (8-20 µg Ni/L). On the other hand, high Ni concentrations (> 200 µg/L) have an opposite (antagonistic) effect. The dose level-dependent effect of the mixtures described above was not reproducible in other experiments. In general the results indicate that a high tolerance to Cu (e.g. Ni) does not necessarily mean that this chemical is harmless. It is important to test low (environmentally realistic) concentrations in mixture studies. If only a more conservative Cu/Ni experimental design would have been assessed (e.g. NOEC, EC10...), the synergism at low Ni concentrations would have been missed. For risk assessment purposes: the CA mixture using the 20°C toxicity of Cu with different antagonistic interactions were observed. CA and IA cannot be used to predict the effect of Cu/Ni mixtures because synergistic effects occur at low concentrations.

206 Does chronic toxicity of Cu and Zn to Daphnia magna depend on temperature?
C.S. Pereira, Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Ghent, Belgium; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Ghent, Belgium; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research Department of Biology, K.A. De Schamphelaere, Ghent University (UGent) / Laboratory of Ecotoxicology and Ecological Risk Assessment (END-ER), Ghent University, Ghent, Belgium.

The aim of the present study was to assess the effects of metal stressors. The main goal of this research was to determine if chronic metal toxicity to Daphnia magna depends on temperature, since few studies are available about the influence of temperature on metal toxicity on Daphnia and temperature acclimation is often not considered. In this study, the chronic toxicity of copper (Cu) and zinc (Zn) on Daphnia magna at 15°C, 20°C, and 25°C was studied. Four clones from a single population of D. magna were acclimated at the same temperature that the 21d-Daphnia magna reproduction tests for Cu and for Zn were performed. 15°C, 20°C, and 25°C. D. magna clones present different sensitivities to Copper and Zinc. At 20°C the 21 days EC50 values for Cu varied between 40 and 51 µg Cu (dissolved/L). For Zn at 20°C the 21 days EC50 values varied between 114 to 153 µg Zn (dissolved/L). Zinc toxicity to D. magna depended on temperature. For total reproduction the 21 days EC50 values for Cu were lower at 15°C and higher at 25°C compared with 20°C, 1 fold variation. For Cu the 21 days EC50 and EC10 values presented on average a 1.4 and 1.5 fold of variation between 15 and 25°C, respectively. Compared with the values at 20°C the 21 days EC50 for Cu were lower at 15°C and similar at 25°C. Therefore the highest toxicity of Cu on D. magna was observed at the lowest temperature 15°C. The 21 days EC10 values for Cu at 20°C and 25°C were higher than the 21 days EC50 at 15°C. However, for Zn this was not observed. Even then, these results raise concerns since the temperature dependency of Cu toxicity on D. magna may not be the same for all species. At 15°C. Our results indicated that chronic toxicity of copper and zinc on Daphnia magna depends on temperature. Cu and Zn chronic toxicity on D. magna were higher at a lower temperature. Therefore the influence of temperature on metal toxicity should be considered in models like the Biotic Ligand Model (BLM).

207 Effects of climate change on the toxicity of metal-polluted soils to Enchytraeus crypticus
M. González-Alcaraz, Faculty of Earth and Life Sciences, VU University / Ecological Science; E. Tsitsiou, Faculty of Earth and Life Sciences VU University / Dept of Ecological Sciences; R. Wieldraaijer, R. Verweij, Faculty of Earth and Life Sciences VU University / Ethical Committee of Ecological Science; C. van Gestel, VU University Amsterdam / Ecological Science

The aim of the present study was to assess the effects of climate change (temperature and soil moisture content) on the toxicity of metal-polluted soils. Bioassays with Enchytraeus crypticus (a soft-bodied Oligochaete living in contact with the soil and consuming contaminants in soil) were performed using soils polluted by metal mine wastes with different characteristics (mine tailing, forest soil) and high pH (7.44). The soil from the woodland had a sandy loam texture, neutral pH (7.44), high electrical conductivity (1.22 mS cm⁻¹), low content of organic carbon (0.07% C, 0.08% O), and cation exchange capacity (CEC) (10.00 cmol kg⁻¹). The forest soil showed a clay loam texture, neutral pH (7.24), low EC (0.03 mS cm⁻¹), high content of OC (4.69%) and high CEC (28.2 cmol kg⁻¹). The soil from the woodland had a loam texture, slightly acidic pH (6.04), high EC (2.43 mS cm⁻¹), low OC content (2.52%) and low CEC (13.1 cmol kg⁻¹). The three soils had high concentration of total metals (in mg kg⁻¹): Cd-16-33, Cu-66-177, Pb-5500-6500 and Zn-8000-9800. The undiluted soil from the mine tailing showed high concentrations of available (extracted with 0.01M CaCl₂) Cd (76.70 µg kg⁻¹) and the watercourse soil of Pb (807 µg kg⁻¹) and Zn (31020 µg kg⁻¹). No effect was observed on enchytraeid survival (average survival >80%). Reproduction was the most sensitive endpoint, and it was reduced by 65-98% compared to the control (Lafa 2.2 soil in the watercourse soil (lower pH, higher salinity and higher available metal concentrations). In this soil, EC50 and EC10 significantly decreased with decreasing soil moisture content. In the forest soil, reproduction was reduced by >50% compared to the control. The present study shows that lowering soil moisture content has the strongest effect on the toxicity of metal-polluted soils. The toxicity of different soils polluted by metal mine wastes under a climate change scenario will depend on soil properties (e.g. pH, salinity) and available metal concentrations.

Plastics in the Aquatic Environment: Mechanisms and Implications (I)

208 Microplastics in the Arctic
J. Albers, Galway-Mayo Institute of Technology / Marine and Freshwater Research Centre; V. Torelli, National Institute of Oceanography and Experimental Geophysics; I. O'Connor, R. Officer, GalwayMayo Institute of Technology

Plastics in the Aquatic Environment: Mechanisms and Implications (I)

209 Waste water treatment plants (WWTP) as a source of microplastics in the aquatic environment
F. Murphy, University of the West of Scotland / School of Science; C. Ewins, University of the West of Scotland / Institute of Biomedical and Environmental Health Research; R. Brennan, Irish Centre for Environmental Toxicology (ICET) / Institute of Biomedical and Environmental Health Research

Waste water treatment plants (WWTP) as a source of microplastics in the aquatic environment

210 Microplastic contamination in an urban area: case of the Greater Paris
R. Dijos, LEESU laboratoire eaux environnement et systèmes urbains; J. gasperi, LEESU / Leesu; N. Furmanski, Unilever / Direction du Développement et de la Prospective; M. Saad, Université ParisEst LEESU UMRMA UPECC ENPC Agroparistech UPEMLV; B. Tassin, Paris-Est University / LEESU laboratoire
Microplastics have been defined as particles < 5 mm. While many efforts were deployed for the assessment of marine microplastic pollution, there has been a limited focus on the plastic contamination of continental hydrosystems: microplastic sources and dynamics remain largely unknown. This study investigates the microplastic contamination in Greater Paris, considering the microplastic fluctuations in the atmosphere and in wastewater, and in the receiving water system. These first investigations on urban debris confirm the presence of microplastics in sewage, freshwater and total atmospheric fallout and provide knowledge on the type and size distribution of microplastics in the [100 – 5 000 µm] range. Whatever the urban compartment considered, microplastics were indeed observed. For the first time, microplastics, mostly fibers, were sampled in atmospheric fallout (29–280 particles/m³/day). High levels of fibers were also found in wastewater (260–320 x10⁶ particles/m³). In treated effluents, the contamination significantly decreases to 14–50 x10⁶ particles/m³. No millimetric fiber was observed in treated effluent attesting to their removal. In river Seine, two sampling devices are used to collect both large and small microplastic particles: i) a plankton net (80 µm mesh size) and ii) a manta trawl (330 µm mesh size). Sampling with the plankton net shows a predominance of fibers with concentrations ranging from 3 to 108 particles/m³. A greater diversity of both microplastic shapes, sizes, and types is encountered during manta trawl sampling but at much lower concentrations (0.28–0.47 particles/m³). This combined approach could be relevant and implemented in future studies to provide an accurate overview of microplastic distribution in freshwater.

211 Microplastics in Swiss surface waters, and going upstream: nature, concentrations, interaction with pollutants E. Faure, C. Demars, EPFL, Swiss Federal Institute of Technology in Lausanne / Central Environmental Laboratory; O. Wieser, EPFL; M. Kunz, Federal Office for the Environment; L. De Almeida, EPFL Polytechnique Fédérale de Lausanne / Central Environmental Laboratory Marine microplastic (< 5 mm) water pollution has met growing public and scientific interest in the last years. The situation in freshwater environments remains largely unknown, although they appear to play an important role as part of the origin of marine pollution. Apart from the physical impacts on biota, chemical effects are to be expected as well, especially with smaller particles. This study aimed at assessing the situation for the Swiss Lakes Geneva, Constance, Neuchâtel, Maggiore, Zurich and Brienz, and identifying potential impacts. Lake surface transects, a few rivers as well as urban runoff outlets and WWTPs have been sampled using a floating manta net, and beach sediments have been analysed. Photos have been collected (fragments were obtained from fibres, films, foams) and composition (PP, PE, PS, etc.). Fish and water birds have been dissected to assess their potential exposure, and analyses of the hydrophobic micropollutants adsorbed to the microplastics as well as some potentially toxic additives they contain have been conducted. It appears that all lakes are affected by this pollution, microplastics of all types and diverse composition having been found in all samples. Birds and fish are prone to microplastic ingestion, and all the tested chemicals (both adsorbed micropollutants and contained additives) were found above the detection limit, and often the quantification limit. The sources and their respective contribution need to be confirmed and quantified, and the ecotoxicological impacts also need further investigation. Other questions remain open, including the transport and fate of the plastic particles in the environment.

212 Combined effects of UV exposure and mechanical abrasion on microplastic fragmentation by polymer types Y. Song, Oil and POPs research group; W. Shim, S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; M. Jung, Korea Institute of Ocean Science and Technology / oil and POPs research group OPRG; G. Han, Oil and POPs research group; M. Rani, S. Jung, Korea Institute of Ocean Science and Technology Fragmented secondary microplastic particles account for the majority of microplastics in the field and have various origins, which makes proper control to be needed. Scientific evidence is, however, far from comprehensive, and this hypothesis is not tested yet. Therefore, effects of UV irradiation and (or) mechanical abrasion on fragmentation of polyethylene (PE), polypropylene (PP) and expanded polystyrene (EPS), the top three polymer types in marine debris monitoring study, was evaluated in laboratory condition. The apparent surface damage of PE and PP pellets and EPS spherules exposed to UV for 180 d was observed by SEM, FT-IR and naked eyes. The surface damage of PE was faster than PP. After 120 d, the cracks on the surface of PE could be identified by naked eyes, while, after 60 d, the weakened PP was embrittled and easily broken down and fine cracks appeared. In case of EPS, yellow color appeared on 60 d after UV exposure and the surface was embrittled and started to create the fine particles on the surface. In the only mechanical abrasion experiment for 60 d, the average fragmented particles per bottle produced from PE, PP and EPS were 174±51, 214±14 and 57,339±7,504, respectively. In combination of UV exposure and subsequent mechanical abrasion for 60 d, the mean fragmented particles from PE, PP and EPS were 73±49, 1,442±123 and 723±134, respectively. In case of PE, 60 days of exposure to UV was not enough long to reach at the critical point for photooxidation causing physical alteration in PE. In the PP, number of fragmented particles produced from combination of UV exposure and mechanical abrasion. Because of UV exposure, the occurrence of chemi-crystallization led to embrittlement of PP. The final size of the EPS spherules after combined experiment was smaller than that of only mechanical abrasion experiment, while number of fragmented particle showed reversed results. It is not clear whether visible white flour of EPS fragments on the surface of spherules were lost during size and weight monitoring process. UV exposure experiment rather than mechanical abrasion is needed to improve knowledge of EPS resistant to mechanical abrasion. Keywords: microplastics, fragmentation, mechanical abrasion

213 Plastic particulate pollution: a multi-scale pollution f. lagarde, Institute of molecules and materials of Le Mans / Institute of Materials and Molecules of Le Mans IMMM UMR CNRS; P. Daniel, University of Le Mans / Insitut des Molecules et Materiaux du Mans IMMM Plastic pellets, fibers and fragments originating directly from industry and domestic sewers are classified as primary source of plastic pollution. The secondary source of these particles results from fragmentation of pellets, straws and bottles due to long time in the environment. At the moment, despite the worldwide distribution of plastic fragments, the global load of plastic on the open ocean surface was estimated to be far less than expected. One of the serious hypotheses regarding the "missing" plastic is that the fragmentation processes may finally lead to sub micrometric fragments. However, while data on microplastics presence in the environment increase rapidly, smaller sub-micrometric fragments could not be quantified so far. Indeed, studying nano fragments requires very specific analytical tools and procedures. Studies on all fragments of plastic, from nano to milli-size, are now of major importance to understand the sources and fate of plastic pollution. In this work, High Density Polyethylene (HDPE) and polypropylene (PP) films were fragmented by high-energy milling in order to generate fragments. The milling was processed at liquid nitrogen temperature in order to decrease elastic properties of the polymers as, after long time in the environment, polymers become more brittle before fragmentation. Generated fragments were characterized by laser granulometry and found to be mostly composed of micro-sized fragments with average sizes depending on the milling conditions but also on the polymer type. This work suggests that, depending on the polymer type, from 20 to 70% of the particles obtained from fragmentation of a plastic item are smaller than 330 µm, which is the size mesh the most often used to collect environmental samples. In all cases, nano-scale particles were detected as only a few percentage (less than 1%). However, after appropriate sieving, the use of several techniques dedicated to nano-scale analysis revealed that nano fragments were present. We will present here the results of these techniques, highlighting the different perspectives on nano fragments of plastics in aquatic media. This work is a first insight in the understanding of plastic fragmentation processes with a first attempt to determine the sizes of particles that fragmentation may generate. It emphasizes that there is a urgent need to sample and characterize microplastics under 330 µm and nanoplastics to determine the "real" levels of pollution in the environment.
Use of polar passive sampling in compliance with WFD monitoring networks g. pouluer, Iersta / Unité de recherche REBX; S. Lisalde, Université de Limoges / Groupe ment of recherche eau sol environnement; A. Charriaou, Labaoratoire GRESE, Université de Limoges; R. Buzier, Université de Limoges / Groupement de recherche eau sol environnement; F. Delmas, Institut national de recherche en sciences et technologies pour l’environnement et l’agriculture; A. Moreira, Iersta / Unité de recherche REBX; G. Guibaud, Université de Limoges / Groupe ment de recherche eau sol environnement; N. Mazella, Iersta.

This contribution discusses the possible application of POCIS data in the regulatory monitoring programme for pesticides. It focuses on one of the main requirements of the WFD, i.e. determination of uncertainty, a key factor for all monitoring programmes. It is assumed that if this parameter is known, POCIS data could be used to complement or replace grab water samples in the context of operational and surveillance monitoring. A confidence interval related to the POCIS data was evaluated and we concluded that it might be up to 138 %, which is higher than European union requirements. However, this issue was adequately counterbalanced by lowering the limits of quantification, as well as a gain of temporal representativeness. Comparison with data from the regulatory monitoring program from French Water Agency showed that the POCIS is already suitable for both operational and investigative monitoring. The sampled fraction issue, and then compliance with Environmental quality standards, was also addressed. We confirmed that POCIS samples only the dissolved fraction and showed that for compounds like atrazine, desethylatrazine and metolachlor, the POCIS concentration is equivalent to the total water concentrations. Finally, an innovative scenario was proposed: passive sampling (rocky mountain) with the framework of regulatory monitoring programs, was suggested.

216 Suitability of Chemcatcher® passive samplers for the comprehensive screening of micropollutants in rivers C. Suquet, Eawag / Uebersee; F. Vermeersen, Eawag / Dept of Environmental Toxicology; H. Singer, Eawag / Aquatic Research; S. Christian, Eawag / Dept of Environmental Chemistry; J. Hollender, Eawag / Environmental Chemistry

The goal of our study was to determine the suitability of passive sampling for a comprehensive screening of a very large number of polar to semi-polar micropollutants that are occurring in streams and rivers. To test this, a field study was carried out where 45 Chemcatcher® passive samplers were deployed, for two week periods, in five agriculturally and urban influenced Swiss rivers from March to July 2012. During the same time periods, two-week time-proportional composite water samples were taken for comparison. The investigated 322 substances – pesticides, pharmaceuticals, industrial chemicals and their transformation products with rivers physico-chemical properties (logKow <3 to 5, neutral, anionic, cationic, and zwitterionic species) – were analyzed by liquid-chromatography high-resolution tandem mass spectrometry. Results showed that Chemcatcher® passive samplers are perfectly suited for the qualitative screening of polar micropolllutants. The number of detected substances was similar for both sampling methods (204 for passive samples vs. 207 for composite water samples), limits of quantification were comparable (ionic sampling (1 ng/L vs. 1.6 ng/L), and the handling of Chemcatcher® in the field and laboratory is fast and easy. The fact that so many different substances accumulate on the sampler also opens the door for a comprehensive screening for suspects or unknowns. In-situ calibrated sampling rates (field Rs) could be determined for 88 compounds, i.e. where a good regression was obtained between water concentration vs. sampled mass on passive sampler did not differ more than 5% from corresponding water concentration. Correlations with highly fluctuating concentrations such as pesticides (R-squared > 0.75 for 93% and 60% of the investigated substances, respectively) showed that this parameter was well described. However, a correlation between field Rs and hydrophobicity (logDow) could only predict Rs with large uncertainties. We conclude that substances with relatively constant river concentrations can be quantified accurately in the field by passive sampling. To do so, substance-specific Rs for these compounds need to be determined. For that purpose, in-situ calibration is a very robust method and the determined Rs can be used in future monitoring studies in rivers with similar environmental conditions.

217 Critical micelle concentrations for different surfactants measured with polycarboxylate-coated SMF fibres J. Haftka, University of Utrecht / Institute for Risk Assessment Sciences; P. Schepenisse, Utrecht University; G. Oetter, BASF; G. Hodges, Unilever Research / Safety and Environmental Assurance Centre; J.L. Hermens, Utrecht University / Institute for Risk Assessment Sciences

The determination of phase-separated properties of neutral organic substances is important to predict partitioning or adsorption to environmental phases of interest. However, surface-active substances are amphiphilic molecules with both a hydrophobic alkyl chain and a hydrophilic head group. The amphiphilic nature of surfactants drives the formation of micelles at the critical micelle concentration (CMC). The CMC value is usually determined by measurement of electrical conductivity (S) or surface tension. The CMC value can also be determined directly by the measurement of surfactant monomer concentrations using solid-phase microextraction (SPME) fibres. Because only freely dissolved concentrations of surfactant monomers are measured, the CMC is derived when the concentrations in the fibre reach a plateau in the fibre-water sorption isotherms. In this study, the determination of CMC for a wide selection of surfactants is evaluated with SMF fibres. The selected compounds covered non-ionic, anionic, cationic, and amphoteric surfactants from different compound classes. The CMC values for all surfactants were derived when the concentrations in the fibre became constant. The determined CMC values were in close agreement to the values reported in literature. In the linear phase of the fibre-water sorption isotherm, the slope of the
Monitoring and modelling-based approaches for identification and prioritisation of hazardous emerging pollutants in European freshwater resources

220 Second review of the Priority Substances list under the Water Framework Directive
R.N. N. Carvalho, European Commission - Joint Research Centre / Institute for Environment and Sustainability; L. Ceriani, European Commission DG Joint Research Centre / Institute for Environment and Sustainability; I.S. A. Ippolito, EFSA - European Food Safety Authority / Institute for Environment and Sustainability; P. Marinov, JRC, EC / Institute for Environment and Sustainability; N. Adibi, CILe - Plateforme avniR / Institute for Environment and Sustainability; L. Letteri, European Commission - Joint Research Centre / Institute for Environment and Sustainability

The European Water Framework Directive (WFD) currently lists 45 Priority Substances (PS) identified as Priority Hazardous Substances (PHS), which have been selected amongst those which present a significant risk to or via the aquatic environment. The substances on this list should not exceed the respective environmental quality standard (EQS), i.e. a concentration that is considered safe to aquatic organisms or to humans via the aquatic environment. Under Article 16 (4) of the WFD, last amended by Directive 2013/39/EU, the Commission is required to review the list of PS at least every six years. The second review of the PS list has started in 2013 and will evaluate substances suspected of posing a risk to the environment following a dual approach i.e. a monitoring- and a modelling-based exercises. The monitoring-based exercise takes advantage of the available monitoring data gathered in Europe under different official monitoring programmes or research projects. Substances with sufficient monitoring data from a representative number of European countries are evaluated in terms of the Spatial, Temporal and Extent of exceedance (STE approach) of the Predicted No Effect Concentration (PNEC) of that substance. By contrast, the majority of substances in the initial list do not have sufficient monitoring data and therefore it is more difficult to estimate the exposure and the risks to the environment. Therefore a modelling-based exercise is run by first screening the substances for the fulfillment of several exposure and hazard criteria and secondly, a modelling-based risk assessment is done for relevant compartments and receptors. The estimation of predicted environmental concentration (PEC) and PNEC makes use of available information in the literature regarding the tonnage, use pattern, fate and effects of each substance, while in the absence of such information, different models are used to fill the knowledge gaps. Finally, the substances are ranked for prioritisation based on the highest of the calculated risk quotients (PEC/PNEC). The prioritization exercise is presented and illustrated with a few case studies.

221 A proposal for an chemical assessment concept for the protection of raw water resources under REACH
M. Neumann, Federal Environment Agency (UBA) / Section IV - Chemicals; M.A. Schwarz, FoBiG GmbH; D. Sättler, Federal Environment Agency UBA / IV Chemicals; J. Ottmanns, FoBiG GmbH; L. Vieker, Federal Environment Agency / Section IV - Chemicals; F. Kablerlah, FoBiG GmbH

Chemical substances and their uses that fall within the scope of the REACH Regulation (1907/2006 EG) have to be registered at ECHA in Helsinki. Registrants are requested to ensure a high level of protection of human health and the environment. By doing so, industry guarantees the safe use of chemicals throughout the whole life cycle. Raw water resources for the production of drinking water need a high level of protection. In Europe drinking water is obtained mainly from groundwater, reservoirs or river bank filtration. If the environmental compartments are exposed to hazardous chemicals a contamination of drinking water is possible. In the last decade the fate and behaviour of polar substances has been investigated both scientifically and from a regulatory perspective. The finding is that their intrinsic hazard potential is maximised if they are at the same time persistent in the environment and mobile in the water cycle. Once emitted, these substances remain in the aquatic environment and the contamination is irreparable. If in addition they also fulfill the properties of being toxic emissions into water resources should be avoided during the production and downstream use of the substances. Here we present a proposal for an assessment concept of persistent, mobile and toxic chemicals (PMT substances). We adopted the criteria for the identification of persistent substances directly from the Annex XIII of the REACH regulation. In Contrast we developed the M criteria with an extensive modelling approach using the software ECETOC-Target Risk Assessment (TRA, Version 3.6). With this research study a proposal for a practical guide was published. This guide may be used by registrants to assess whether a substance cause a concern for raw water. Acknowledgment This study was conducted as project No. 3712654416 for Environmental Research of the Federal Ministry for the Environment, Nature Conservation, and Nuclear Safety in Germany, in 2012 to 2014

222 Early identification of organic chemicals with a potential for long-term environmental contamination
M. Scheringer, ETH Zuerich; G. Stieger, ETH Zürich / Institute for Chemical and Bioengineering; J. Hollender, A.C. Chaia-Hernandez, Eawag / Environmental Chemicals Characterization with a multimedia ART model for Switzerland; Chemical Natural Resources / Institute for Environment and Agriculture; A. Keller, Agroscope ART; D. Wächter, NABO Swiss Soil Monitoring Network; K. Hungerbuhler, ETH Zurich

There are several thousands of organic chemicals on the market and it is a challenging task to identify those with a potential for long-term environmental contamination. Chemical-intensive economies repeatedly cause contaminations of the environment that mainly lead to long-term global effects. The potential of perfluorinated alkyl substances (PFASs), which have been used since the 1970s but were detected in the environment only after 2000. Because analytical campaigns and monitoring programs can cover only a selection of chemicals, strategies for identifying priority chemicals for analytical measurements are needed. Here we present a procedure that starts from an extensive computer-based screening of chemicals that may have a potential for long-term environmental contamination. This first step is complemented with field sampling of representative soils and sediments and application of analytical methods for identifying organic substances with logarithmic octanol-water partition coefficients (log Kow) between 1 and 8. This procedure is applied to chemicals on the Swiss market; here we report mainly results from the first step. Relevant chemicals were extracted from Swiss customs statistics and product registers. This led to 17'409 individual chemicals. For 9593 (49%) of these, it was possible to retrieve their physicochemical properties and degradation half-lives. 2247 of these substances are halogenated and were selected for further investigation because halogenation facilitates analytical detection (characteristic isotopic patterns). The environmental partitioning of these chemicals was characterized with a multimedia ART for Switzerland; chemicals with 90% or more in soil or sediment were identified (469 substances). This group includes a wide range of chlorinated, chlorinated and brominated aromatic and aliphatic substances, often with highly branched substituents, ether and tertiary amine groups, trifluoromethyl groups, phosphate ester groups etc. In the second step of the procedure, an analytical screening based on extraction of soil and sediment samples by pressurized liquid extraction and clean-up by liquid-liquid partitioning is performed and followed by liquid chromatography coupled to high-resolution mass spectrometry with electrospray ionization. Suspect screening of exact masses of chemicals possibly covered by this analytical method is performed and several contaminants have already been identified and confirmed by reference standards.

223 Novel toxic pressure assessment for data poor substances by means of ecotoxicological concern level estimation
L. Poehling, RIVM / Centre for Sustainability Environment and Health; K. Kramer, Mermayde; D. van de Meent, RIVM / DMG; I. Ottenbros, Utrecht University; D. De Zwart, RIVM / Centre for Sustainability Environment and Health

This presentation considers the challenging risk assessment of data-poor next to data-rich substances, describing and exploring an innovative, distributions-based ecotoxicity assessment approach for the former. Within the EU FP7 project SOLUTIONS, a solution-focused approach has been designed to address the specific concern of variable mixture composition and future emissions. In a modeling-measuring combination, innovative approaches will be designed and evaluated for risk and impact assessment. This presentation focuses on risk modeling of mixtures of data rich and data poor substances together. Classically, toxic pressures for data rich compounds (a low fraction of all compounds) are derived using Species Sensitivity Distributions (SSDs), based on compound-specific ecotoxicity data. Addressing the challenge of looking at potentially all the other compounds (the big fraction of compounds), we develop and test a novel distribution-based method, to evaluate the (net mixture) risks of data poor substances. The novel method was inspired by the concept of Threshold of Toxicological Concern (TTC) assessment. The method is applied, starting from
Danube-catchment emission data. When validated against (SOLUTIONS) effect data, the method may have implications for chemical footprint assessments, which in turn relate to (planetary) boundary definition and sustainable use of chemicals.

224 An innovative integrated system of models and databases in support to the prioritisation of emerging contaminants on a European scale J. van Gils, DELTARES; D. van de Meent, RIVM / DMG; I.T. Cousins, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; D. Georgieva, Laboratory of Mathematical Chemistry / Laboratory of Mathematical Chemistry; A. Kortenkamp, Brunel University; D. De Zwart, RIVM / Centre for Sustainability Environment and Health; C. Lindim, ITM Department of Agricultural Environment; S. Los, Stockholm University / Laboratory for Applied Environmental Chemistry. Emerging pollutants may be posing a significant ecological and human health risk through their presence in water bodies. We do not know enough of them however, to assess and manage this risk. The EU FP7 project SOLUTIONS addresses this issue. SOLUTIONS combines monitoring and modelling-based approaches towards the prioritisation of emerging contaminants. This paper discusses the relevant modelling tools. The specific value of modelling tools is that, after a successful validation for well-studied substances and data-rich basins, the models allow extrapolations to "new" substances and other basins for which data are lacking. The SOLUTIONS integrated model system consists of four interlinked components. The risk modelling component follows a common tiered framework for human and ecological risk assessment. Specific ecotoxicological risk assessment will address mixtures effects on aquatic ecosystems. The risk modelling is driven by integrated components for emission and fate & transport modelling, supplemented by a component providing new methods for the prediction of partitioning, transformation and toxicity of emerging compounds. The modelling is spatially and temporally explicit and driven by detailed hydrology modelling. The fate & transport component provides mechanistic process-based modelling of a wide range of substances, including polar and ionic organic substances. The emission modelling component builds on collected and estimated market and use volumes for chemicals by combining data from existing registration frameworks, together with information on specific uses and releases of chemicals. The model framework relies on external data specifying the morphology, hydrology, geology and relevant socio-economic aspects on a European scale. The implementation of the model framework is planned over a five year period (2013-2018). The work so far has focused on (1) creating a flexible spatial schematisation, (2) making available the necessary hydrology forcing (water fluxes carrying emerging contaminants with them), (3) modelling particle fluxes (carriers of emerging contaminants) and (4) implementing first versions of the various submodels. The relevant parameters are the source term and its spatial distribution. The integrated set of models and databases. We are well under way towards an important milestone, being the first model evaluation for around 300 chemicals using the 3rd Joint Danube Survey field data.

225 Estimating the major sources of PFOS and PFOA to the Danube River catchment C. Lindim, ITM Department of Applied Environmental Science; I.T. Cousins, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; J. van Gils, DELTARES. Among perfluoralkyl substances, perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) are of special concern because they are persistent, bioaccumulative, toxic and globally distributed in the environment. The aquatic compartment is the main sink and transport media for these compounds. However there is no consensus regarding the dominant sources of PFOA and PFOA impacting aquatic ecosystems. While emissions from WWTPs are reportedly a major source, strong correlations were also found between population and PFOA and PFOA levels in water regardless of efficient treatment coverage or efficiency. In this work we use the Danube basin as case study to investigate dominant emission sources contributing to riverine loads of PFOA and PFOA. We test three hypothesis: 1. Human population alone can explain PFOA and PFOA levels in river water. 2. Human population combined with WWTP treatment can explain PFOA and PFOA levels in river water. 3. Human population combined with consumer wealth can explain PFOA and PFOA levels in river water. A fate and transport model with subcatchment resolution was used to simulate PFOA and PFOA concentrations in the river Danube for the three different emission hypotheses tested. Model predictions were then compared with measured values. It was found that PFOA emissions based only on population (hypothesis 1) or on population plus WWTP contributions (hypothesis 2a) are not able to explain levels and spatial distribution of PFOA measured in the Danube River. For PFOA, we found that emissions have spatial variations that can be reasonably well attributed to GDP differences. PFOA emissions are therefore associated with consumption and disposal of PFOA-containing products. For PFOA our results were inconclusive about the dominant contributions to emissions but they point out that where industrial contributions exist or once existed their regional importance largely surpasses contributions from humans per se for locations with high populations.

Nanoparticle (NP) speciation and its consequences for NP environmental fate and effects (I)

226 Are current approaches suitable to describe the environmental fate of nanopesticides? M. Kah, University of Vienna / Department of Environmental Geosciences; A. Weniger, University of Vienna; T. Hofmann, University of Vienna / Department of Environmental Geosciences. Research into nanotechnology applications for use in agriculture has become increasingly popular over the past decade. We first carried out a comprehensive literature review [1] with the primary aim (i) to explore the application of nanotechnology within the pesticide formulation sector, (ii) to identify possible impacts on environmental fate, and (iii) to analyse the suitability of current exposure assessment procedures to account for their novel properties. Investigations into the environmental fate of nanopesticides remain scarce, and current approaches, which considers the soluble portion of organic substances to be of importance, may not adequately describe the nano behaviour that some nanopesticides will exhibit after application [2]. The latest trends in research [3] indicate that polymer-based nanoformulations seem to have the greatest potential for further development. Experiments were thus carried out on a series of polymer-based nanopesticides representing a range of properties. We considered two active ingredients covering extreme scenario for sorption and degradation characteristics and polymer nanocarriers comprising a range of colloidal characteristics (e.g., size distribution, electrostatic vs steric stabilization). The application of regulatory protocols to determine sorption and degradation in two agricultural soils (OECD guidelines) confirmed that a nanoformulation can affect the fate of an active ingredient [3]. Conclusions about exposure assessment should be made with some reserve as currently used protocols were designed to assess the fate of solutes, and may not adequately described the "nano" behaviour of active ingredients associated with nanocarriers. Results from additional tests looking into release profiles and transport behaviour in porous media, serve as a useful basis to discuss the (in)adequacy of current approaches, and make recommendations for a more robust regulatory evaluation of environmental risks. [1] Kah et al. (2013). Critical Reviews of Environmental Science and Technology. 43, 1823-1867; [2] Kookana et al. (2014) J. Agric. Food Chem. 62, 4227-4240; [3] Kah and Hofmann (2014) Environ. Intern. 63, 224-235; [4] Kah et al. (2014) Environ. Sci. Pollut. Res. 21, 11699-11707.

227 Aggregation of engineered nanoparticles in the natural environment: is the process reversible? S.R. Thompson, Depment of Physics Environment Department; J. Yuan, University of York / Physics; A. Boxall, University of York / Environment Department Engineered nanoparticles (ENPs) are increasingly being used in a wide range of product classes. The increase in use of ENPs will inevitably lead to increased emissions to the natural environment so it is important to develop an understanding of the environmental fate, behaviour and effects of ENPs. Following release to the environment, ENPs can aggregate depending on the environmental conditions; consequently aggregation is one of the key processes that is included in environmental exposure modelling exercises. However, in these exercises, it is typically assumed that aggregation proceeds in one direction and that the process in non-reversible. This is an important assumption, because usually the primary unaggregated particles will have significantly greater ecotoxicity compared to the aggregated systems. This study examines the validity of this assumption, by exploring whether changes in environmental parameters including pH, temperature, and kinetic environment could result in disaggregation of ENP aggregates. Results suggest that environmentally relevant changes in environmental parameters can result in disaggregation of selected ENPs. Existing exposure modelling approaches may therefore need adapting to account for this reversibility of the process.

228 Fate and effects of silver nanoparticles in aquatic indoor microcosms C. Wasmeuth, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; C. Schaefer, Fraunhofer-Institut / Ecotoxicology; H. Ruedel, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Environmental Monitoring; T. Klawonn, Fraunhofer IME Institute for Molecular Biology and Applied Ecology. Research into nanotechnology applications for use in agriculture has become increasingly popular over the past decade. We first carried out a comprehensive literature review [1] with the primary aim (i) to explore the potential application of nanotechnology within the pesticide formulation sector, (ii) to identify possible impacts on environmental fate, and (iii) to analyse the suitability of current exposure assessment procedures to account for their novel properties. Investigations into the environmental fate of nanopesticides remain scarce, and current approaches, which considers the soluble portion of organic substances to be of importance, may not adequately describe the nano behaviour that some nanopesticides will exhibit after application [2]. The latest trends in research [3] indicate that polymer-based nanoformulations seem to have the greatest potential for further development. Experiments were thus carried out on a series of polymer-based nanopesticides representing a range of properties. We considered two active ingredients covering extreme scenario for sorption and degradation characteristics and polymer nanocarriers comprising a range of colloidal characteristics (e.g., size distribution, electrostatic vs steric stabilization). The application of regulatory protocols to determine sorption and degradation in two agricultural soils (OECD guidelines) confirmed that a nanoformulation can affect the fate of an active ingredient [3]. Conclusions about exposure assessment should be made with some reserve as currently used protocols were designed to assess the fate of solutes, and may not adequately described the "nano" behaviour of active ingredients associated with nanocarriers. Results from additional tests looking into release profiles and transport behaviour in porous media, serve as a useful basis to discuss the (in)adequacy of current approaches, and make recommendations for a more robust regulatory evaluation of environmental risks. [1] Kah et al. (2013). Critical Reviews of Environmental Science and Technology. 43, 1823-1867; [2] Kookana et al. (2014) J. Agric. Food Chem. 62, 4227-4240; [3] Kah and Hofmann (2014) Environ. Intern. 63, 224-235; [4] Kah et al. (2014) Environ. Sci. Pollut. Res. 21, 11699-11707.
229 The fate of nanomaterials at the river/ocean frontier using large mesocosms: the case study of silver nanoparticles

E. Pelletier, University of Quebec, Rimouski / ISMER/UQAR; G. Bardaxiglou, University of Quebec at Rimouski / ISMER/UQAR; M. Barthe, Université du Québec Rimouski; P.G. Campbell, Université du Québec, INRS / INRS Eau Terre Environnement; I. Desbiens, University of Quebec, Rimouski / ISMER; C. Fortin, Université of Quebec / Centre Eau Terre Environnement; I. Gagné, Université du Québec Rimouski; C. Gagnon, Environment Canada / Centre Saint-Laurent; J. Gagnon, Université of Quebec in Rimouski; C. Guilleux, Université du Québec INRS; K. Lemarchand, Université du Québec Rimouski; M. Millour, Université du Quebec Rimouski; C. Roleau, Peches et Oceans Canada; G. Triffault-Bouchet, Centre expertise en analyse environnementale du Quebec / Ecotoxicologie et éé critique in the seawater layer [29 PSU] at bottom. The freshwater layer contained engineered silver nanoparticles (ESN, 20 nm mean diameter, citrate coated), dissolved and particulate organic matter (POM), suspended and a diatom and clay. The unfiltered seawater layer contained pelagic microorganisms and several macro-benthic species. Each mesocosm was sampled at different depths for physical, chemical and biological monitoring over a period of 6 weeks. The turnover time for the water mass in each mesocosm was about 36 h, allowing enough time for some slow reactions such as adsorption/desorption equilibrium on particulate matter, auto- and hetero-aggregation and sedimentation to occur. The percentage of dialyzable silver (100-500 Da) in the freshwater layer increased from about 5 to 15% in the first three weeks, indicating a slow dissolution of ESN and a possible trapping of dissolved Ag in the fresh water layer. Dissolution was shown to increase with increasing salinity. The main results indicate a strong retention of AgNPs in the halocline layer with a very limited transport of settling particles through the halocline or the halocline layer. Macro-benthic organisms (black mussels, clams, amphipods, sea urchins and winkles) held in the seawater layer for weeks did not accumulate significant amounts of silver. However, blue mussels exposed to the mesocosm effluent (mixed waters with ESN) showed a strong bioaccumulation of silver, confirming the bioavailability of AgNP to marine species in contact with the mixing layer. ESN hetero-aggregated with clay and POM represent underestimated risks for marine organisms (mainly larvae and juveniles of marine invertebrates and fish) swimming and feeding in the halocline layer in coastal waters.

230 A comparative study of the influence of water chemistry (humic acid, and hardness) on silver nanomaterial toxicity to model organisms Lumbriculus variegatus and Danio rerio

C.C. Liddle, F.G. Lara, H.J. Jonston, T.B. Henry, T.F. Fernandes, Heriot-Watt University / School of Life Sciences

Given the widespread use of silver nanomaterials (Ag NM), their release, whether accidental or intentiona l, into the environment is inevitable. At this point, Ag NPs show a high rate of aggregation and sedimentation to occur. The percentage of dialyzable silver (100-500 Da) in the freshwater layer increased from about 5 to 15% in the first three weeks, indicating a slow dissolution of ESN and a possible trapping of dissolved Ag in the fresh water layer. Dissolution was shown to increase with increasing salinity. The main results indicate a strong retention of AgNPs in the halocline layer with a very limited transport of settling particles through the halocline or the halocline layer. Macro-benthic organisms (black mussels, clams, amphipods, sea urchins and winkles) held in the seawater layer for weeks did not accumulate significant amounts of silver. However, blue mussels exposed to the mesocosm effluent (mixed waters with ESN) showed a strong bioaccumulation of silver, confirming the bioavailability of AgNP to marine species in contact with the mixing layer. ESN hetero-aggregated with clay and POM represent underestimated risks for marine organisms (mainly larvae and juveniles of marine invertebrates and fish) swimming and feeding in the halocline layer in coastal waters.

231 Physicochemical properties of AgNP influencing bioaccumulation and toxicity in Lumbricus rubellus

S. Sakama, Alterra Wageningen University and Research Centre / Toxicoology/Alienology, A. Undas, RIKILT Inst. Of Food Safety WUR / Business Unit Contaminants - Toxins; J. Piella, Institut Catalá de Nanociencia y Nanotecnología (ICN2); R. Peters, RIKILT / Contaminants; V. Punctes, Institut Catalá de Nanociencia y Nanotecnología (ICN2); N.W. van den Brink, Wageningen University / Dept of Toxicology

In this study, our purpose was to investigate the effect of size, surface coating and associated charge of silver nanoparticles (Ag NPs) on the bioaccumulation in and toxicity to the red earthworm Lumbricus rubellus. Silver nanoparticles were synthesized to produce three sizes: 20, 35 and 50 nm. These were coated with either chitosan (CHIT), bovine serum albumin (BSA), or polyvinylpyrrolidone-55 (PVP-55) to produce positive, negative and neutral particles respectively. During a 28-day sub-chronic reproduction toxicity test, earthworms were exposed to varying concentrations of the nine (9) AgNPs in wet-skipped soil. To compare the bioaccumulation of Ag NPs to the water phase, the water phase showed that maximum concentration of ionic silver in the water phase were between 60 and 80 ngAg/L, concentration of ESN (positive). Total Ag NM toxicity is expected to reflect a concentration-dependent accumulation in earthworms, with more Ag likely accumulating in tissues from AgNO3 treatment groups. Analyses of Ag speciation in earthworm tissues is underway and will be presented, with specific attention paid to whether quantified particulate or ionic burdens of the earthworms explain toxicity most. The present study provides further evidence of the influence of surface coating (charge) as well as size in driving bioaccumulation and toxicity.

Delving into the planetary boundary concept and issues related to biodiversity and natural resources use in LCA (I)

V. Ross, S. Humbert, Quants; S. Gueye, U. Schenker, Nestlé Research Center; L. Lundquist, N. Espinoza Orias, Nestlé; T. Leheusvita, O. Koski, UPM; R. Taylor, Texas AM University / Trace Element Research Laboratory; P. Oliveira, UPM; Companies and communities are increasingly adopting responsible sourcing practices in their supply chain. However, the benefits of using responsibly sourced products are still difficult to quantitatively capture in the context of life cycle assessment (LCA) in particular with regards to their benefits for biodiversity or ecosystem services. Nestlé, a company aiming at adopting responsible sourcing practices throughout its supply chain, UPM, one of their suppliers and a global leader in sustainable forest management practices, along with Quants, a company expert in LCA, have developed an approach to quantify the ecological benefits of responsible forest management practices using environmental indicators typically used in LCA, including impact on ecosystems/Earth system quality (in PDF.m2.y), land use (in m2.y), and GHG emissions. The aim was to build a solid methodology that can be adopted within an LCA content, the relevant differences between conventional and responsible forestry practices for several case studies, of which semi-natural forest in Finland is presented. The study is for one cubic meter of wood, at mill gate, and encompasses the inputs for forestry management, activities on the logging site, logistics until the mill gate and the differences in energy inputs and outputs for heat recovered from wood residues in the mill. The use of the wood fibre based product and its end-of-life are not considered (considered identical for all types of wood sourcing). Carbon uptake and all GHG emissions are considered. The method for biodiversity accounts for four indicators, native tree species composition, deadwood volume and quality, protected valuable habitats, and forest structure, that are grouped into one indicator between 0 and 1. The results show that responsible practices have consistently lower impacts than conventional practices for all indicators. Wood fibre will replace lignin-based lignin-based products, and wood fibres are similarly exposed to environmental impacts. The results indicate that responsible forestry practices are about half of those for conventional practices. This method can objectively capture the benefits of biodiversity protection in wood fibre production. Companies can use it in
233 Applicability of acoustic diversity to life cycle assessment of land use: a case study of oil palm production in South Sumatra

K. Havashi; V. Dhian, E. No-Last-Name, Indonesian Oil Palm Research Institute

Recent publication of global characterization factors (CFs) for life cycle assessment (LCA) of land use has enabled the assessment of potential impacts of land use on biodiversity. However, biodiversity impact of agricultural management practices calculated using the CFs tends to be dependent only on crop yields and the necessity of establishing more refined CFs was pointed out. In conducting more refined biodiversity assessment, one of the crucial issues is the lack of data especially at the site-specific conditions. Therefore, another research direction is required, in which data collection is implemented within the methodology. A promising approach is the use of simple acoustic monitoring to assess biodiversity. The purpose of this study is to appraise applicability of acoustic diversity to LCA of direct land use impacts. In order to measure biodiversity in different land use (oil palm plantations and natural forests), we made sound recording at the fields and analyzed acoustic files. The Acoustic Complexity Index (ACI) was utilized for quantifying each sound file. The case study was carried out in South Sumatra. Four recording points were determined within and next to the Duwas plantation of the Indonesian Oil Palm Research Institute in two years. In the plantation, the most common in distance between and one point in conservation forest. The results indicate that the ACI illustrates a state of recorded sounds produced by animals, rather than directly indicating the degree of biodiversity. Biodiversity can be explained as the behavior of the states. Therefore, biodiversity is described using scale parameters such as standard deviations rather than location parameters such as means. An implication of the results is that scale parameters can be used as a proxy for quantifying biodiversity, which is the vertical axis in the assessment framework for LCA of land use. Using rapid bioacoustics in LCA of land use is promising, because it can gather site-specific biodiversity data without using much time and cost, although there are limitations due to acoustics, i.e., the data are limited to sounds produced by animals when moving, communicating, and sensing their environment. The next step of this study is justification of methodology through the identification of animal species appeared in sound files.

234 Nature conservation in Life Cycle Assessment - new method and case study with palm oil industry

J.H. Schnaidt; 2-0 LCA consultants

In agriculture and forestry, an important means for mitigating impacts on biodiversity and climate change is nature conservation. However, this is seldom included in life cycle assessment (LCA) and most LCA and footprint guidelines prescribe that off-setting shall be excluded. Obviously, there are good reasons for excluding indirect land use changes, as they can be used as a proxy for quantifying biodiversity, which is the vertical axis in the assessment framework for LCA of land use. Using rapid bioacoustics in LCA of land use is promising, because it can gather site-specific biodiversity data without using much time and cost, although there are limitations due to acoustics, i.e., the data are limited to sounds produced by animals when moving, communicating, and sensing their environment. The next step of this study is justification of methodology through the identification of animal species appeared in sound files.

235 Land use impacts on biodiversity: Results from the consensus-building procedure sponsored by UNEP/SETAC’s Life Cycle Initiative

L. Milia | Canals, UNEP; O. Michelsen, NTNU | Industrial Ecology Programme; R.F. Teixeira, Biology; D.M. Souza, Swedish University of Agricultural Sciences; M. Curran, Institute of Environmental Engineering, ETH Zurich; A. Assumpcio, IRTA

Environmental impacts of production and consumption are increasingly coming into the focus of companies and governments. Life Cycle Assessment (LCA) is one of the most important approaches to quantify environmental impacts of products from cradle to grave. Applied as a decision-making tool and/or as a support to policy development, it makes use of indicators to consider different environmental aspects over a system’s life cycle. With increasing economic globalization, there has been a steady growing need to create a worldwide consensus set of environmental indicators. The UNEP SETAC Life Cycle Initiative has successfully provided a platform for consensus finding in the area of environmental indicators in LCA. A flagship project has now been established by the Life Cycle Initiative intended to run a global process aiming at global guidance and consensus building on a limited number of environmental indicators, including indicators those for assessing impacts from land use (LU) interventions, such as land use change (LUC), on biodiversity. LU/LUC is one of the main drivers of biodiversity loss and degradation of a broad range of ecosystem services. Land competition is very likely to increase in the future and the task to halt biodiversity loss became one of the top global priority issues. LCA has a decisive word to say because of its nature as an aggregator of direct and indirect impacts allows producers and policy-makers to assign responsibility for damages to biodiversity. However, despite substantial contributions to address biodiversity loss in LCA, no clear consensus exists yet on the use of (a) specific impact indicator(s). Given the importance of the environmental pressures on biodiversity and how much LCA can potentially contribute to conservation targets, it is imperative to align the LCA community landscape. The consequent bias thus is calculated in the units—absolute species loss due to land occupation and transformation for six land use types (annual crops, permanent crops, extensive forestry, intensive forestry, pasture and urban), and five taxa (plants, mammals, birds, amphibians, and reptiles) in 804 terrestrial ecoregions. The empirical data for model input parameters was collected through extensive literature review. Uncertainty is assessed using Monte Carlo simulations. While the SAR models account for ecosystem vulnerability, in this study we propose to additionally include species-specific vulnerability score (VS; giving higher weight to species near extinction), as an indicator for global extinction risk. The VS (scaled between 0 to 1) is a function of the geographic range of the species and the IUCN assigned threat level. Globally across all ecoregions, the CF values for a particular taxa and land use type ranged over six orders of magnitude. For a given taxa in an ecoregion, the CFs of different land use types varied by an order of magnitude. For a particular land use type in an ecoregion, the CFs differed by two orders of magnitude across the four taxa. Highest CFs were observed for land use in tropical ecoregions, followed by temperate and boreal areas. The results therefore show that the region where land uses take place is relatively more important than the land use type or taxa when considering species loss. The VS-weighted countryside CFs give absolute instead of a relative measure for biodiversity loss in LCA and can be used by decision-makers to quantify the biodiversity footprint of products with complex supply chains and globally distributed land use flows.

Assessment of risks posed by systemic insecticides to hymenopteran pollinators: from lab via (semi-)field to landscape scale testing (I)

237 Large-scale monitoring of seasonal effects of Clothianidin dressed oilseed rape on pollinating Hymenopterans in Northern Germany: Project aims and project area

F. Heinrichs; A. Russ, Tier3 Solutions GmbH

To protect pollinating insects from challenges of Plant Protection Products (PPP)
and their possible adverse effects have to be assessed. The EFSA Guidance on Risk Assessment of PPPs on bees also suggests an exigency of risk assessment for honey bees at the landscape scale. In compliance with these regulations, Elado®, a commercial seed coating of Oilseed Rape (OSR) containing Clothianidin as active ingredient, was registered in the EU in 2006. The application of Clothianidin was suspended for two years in 2013 and is now subject to extensive re-examination. Therefore, the Large-scale Monitoring of Long-term Effects of Elado® (9g Clothianidin & 2g Beta-Cyfluthrin/kg seed) Dressed Oilseed Rape on Pollinating Insects in Mecklenburg-Vorpommern, Germany™ was initiated. This project aims to investigate possible side effects of Elado dressed OSR seeds on honey bee colonies, mason bees and bumble bees under actual agricultural conditions. The study was conducted in Mecklenburg-West Pomerania (Mecklenburg-Vorpommern) because of the large-scale monitoring project in this state. The agricultural fields in this area are well suited for the project as they represent the typical landscape characteristics and agronomic infrastructure of northern Germany. OSR is cultivated in large-scale of the arable land and Elado-treated OSR varieties are preferentially applied. The farms of this region operate under large commercial models and, thus, enable an efficient cooperation with a manageable number of farmers. Two circular Study Sites of approximately 65 km² each surround the investigated bee hives and provide in total nearly 1.800 ha of OSR crops (27 % of arable land). The project consists of four different pollinator studies performed by different institutions at the same time during OSR blossom: a honey bee, a mason bee and a bumble bee monitoring study, as well as a residue analysis of nectar and pollen from foraging honey bees. The monitoring studies will be presented in this paper. Clothianidin residues in OSR did not cause any detrimental effects on the development of hives and brood of honey bees and bumble bees or the nestling activity and reproduction of mason bees neither during OSR blossom in spring nor thereafter until the end of the study. The pollen composition, infestation with diseases and parasite load was also not affected by the exposure to Clothianidin treated OSR in any of the investigated bee species.

238 Large-scale field study of seasonal effects of clothianidin-dressed oilseed rape on honey bees (Apis mellifera) in Northern Germany

D. Rölke, Institut für Bienenkunde Oberursel Polytechnische Gesellschaft; B. Grünewald, Goethe-Universität Frankfurt am Main / Institut für Bienenkunde; W. Blenau, University of Cologne / Zoological Institute;

We investigated possible side effects of Elado-dressed oilseed rape (OSR) on various pollinating insects in a large-scale monitoring project in Mecklenburg-West Pomerania, Germany in 2014. In this region, OSR is usually cultivated at 25-33 % of the arable land. Honey bees were used for a 2-week-long monitoring study, whereas about 1.1 µg/kg were analysed at 3 out of 6 locations in the treatment site. At all other treatment locations, the concentration of Clothianidin residues was below the Limit of Quantification (LOQ = 1.0 µg/kg). In summary, Clothianidin treated OSR did not cause any detrimental effects on the development of the bumble bee colonies neither on growth of the hive nor on reproduction.

240 Large-scale field study of seasonal effects of Clothianidin dressed oilseed rape on mason bees (Osmia biocnris) in Northern Germany

B. Peters, Tier3 Solutions GmbH

In order to investigate possible long-term side effects of Elado dressed Oilseed Rape (OSR) on different pollinating insects, a large-scale monitoring project was initiated in 2014 in Mecklenburg-West Pomerania, Germany. In this region, OSR is usually cultivated at 25 - 33 % of the arable land. The farms of this region operate under large commercial models and, thus, enable an efficient cooperation with a manageable number of farmers. Two circular Study Sites of approximately 65 km² each surround the investigated bee hives and provide in total nearly 1.800 ha of OSR crops (27 % of arable land). The project consists of four different pollinator studies performed by different institutions at the same time during OSR blossom: a honey bee, a mason bee and a bumble bee monitoring study, as well as a residue analysis of nectar and pollen from foraging honey bees. The monitoring studies will be presented in this paper. Clothianidin residues in OSR did not cause any detrimental effects on the development of hives and brood of honey bees and bumble bees or the nestling activity and reproduction of mason bees neither during OSR blossom in spring nor thereafter until the end of the study. The pollen composition, infestation with diseases and parasite load was also not affected by the exposure to Clothianidin treated OSR in any of the investigated bee species.
field site. Prior to the start of the study worker honeybee brood were collected from colonies and returned to an incubator in the laboratory where the adult bees were allowed to emerge. The emerged bees were ‘tagged’ with RFID tags. Three cohorts of 100 bees (approximately one week apart) were fitted with RFID tags and were introduced to a study colony. RFID readers were fitted to the entrances of the colonies and used to monitor the activity of the tagged bees for the duration of flowering (approx. five weeks). The activity data were analysed to assess any impact of flowering on the site compared to the control sites. In addition samples of plants, flower, pollen and nectar were collected during the exposure phase for residue analysis. Colony assessments were made prior to flowering and then at monthly intervals post exposure until the end of overwintering. Under the conditions of this experiment there was no effect of foraging on thiamethoxam treated oilseed rape on honeybee activity, colony development or honeybee colony survival. Additionally, it is possible to use data generated with this relatively new methodology, to assess behaviour and duration of activity for individual bees.

242 Long-term exposure of honeybee colonies with thiacloprid: a field study R. Siede, Bieneninstitut Kirchhain; L. Faust, Goethe-Universität Frankfurt am Main / Institut für Bienenkunde; M. Meixner, Bieneninstitut Kirchhain; C.D. Maass, B. Grünewald, Goethe-Universität Frankfurt am Main / Institut für Bienenkunde; R. Büchler, Bieneninstitut Kirchhain Thiacloprid is a systemic insecticide which is broadly applied to flowering oilseed rape. Residues can occur in matrices in bee hives from colonies foraging on treated oilseed rape. Thus, a long-term exposure of bees with sub-lethal thiacloprid concentrations is a realistic scenario. Its relevance for the colony health, however, is controversially discussed. To improve our understanding of the potential impact of thiacloprid on bee colonies, a field trial with 30 bee colonies was performed in Germany from 2011 to 2014. All colonies were started simultaneously as shok swarm in July of each year and located in an experimental yard. The colonies were divided into three groups of ten colonies each, with colony and year as replicates. The control colonies (C) were each fed with 25 l of pure sugar syrup, colonies of the treatment groups received syrup spiked with 200 ppb (T200) or 2000 ppb thiacloprid (T2000) between July and October. Colonies were weighted and the colony strengths were estimated in intervals of 21 days according to the Liebefeld method. Dead bees were counted regularly. Infestation rates with Varroa and Nosema were measured. All colonies developed similarly and no differences between the experimental groups were detected. The differences between the groups related to the factor ‘treatment’ in terms of the performance of the colonies were statistically not significant (p>0.05, linear mixed model, spss, v 19). Variances between the experimental years were obvious, but not significant (p>0.05). The incidence rates of colonies with very low losses were similar for all three groups. Before and after winter stored food we extracted from the combs and analyzed for thiacloprid and its metabolite. After winter mean residues were 0.012 mg/kg for C (SEM 0.003, med. 0.006), 0.094 mg/kg for T200 (SEM 0.008, med. 0.096) and 0.634 mg/kg for T2000 (SEM 0.034, med. 0.648), underlining the long duration of the exposure of the bees. Nevertheless, neither winter losses nor health problems of colonies in the spring were obvious. The key message of this study is that real-life thiacloprid doses does not impair colony fitness and survival under field trial conditions.

Science communication - Concepts and activities from past and current research projects

243 Planning of Dissemination Activities in Scientific Research Projects: Strategic Considerations based on Practical Experiences T. Gosch, University of Tuebingen / Institute for Experimental and Clinical Pharmacology and Toxicology. The demand for research projects to develop action plans for dissemination and outreach is increasing. Respective requests are formulated in many funding schemes, particularly in the case of collaborative research projects such as large-scale integrated projects funded by the European Commission. In principle, addressing these issues requires clarification of two important points: - What are the target groups of the dissemination strategy? - What are the expectations (of the funders, the scientists) in the dissemination strategy? The first point is obvious and there is no doubt that different target groups (such as the open public or stakeholder groups) must be addressed by different means. The second point seems to be even more trivial, as the overall goal of any dissemination strategy is to raise awareness of the target groups on the results of the research project. But: be careful, the devil is in the detail. The reasoning that the open public is an important target group, science journalists need to be involved in the process. And the ways how journalists raise awareness was a big source of conflicts in the past (‘mis-representation’ of the project, ‘sensationalism’, etc.). Hence, we are confronted with the ‘how’-question in a traditional area of conflicts between scientists and journalists: how can both groups interact in a way that serves the needs of both of them? This contribution will not give ultimate answers, but formulate suggestions based on practical experiences.

Tutorial Video Series: Using Stakeholder Outreach to Increase Usage of ToxCast Data C. Baehkikian, US EPA ASPH Fellow / National Center for Computational Toxicology; T. Bahadori, American Chemistry Council; R.S. Thomas, The Hammer Institute for Health Sciences / Institute for Chemical Safety Sciences, Center for Genomic Biology and Bioinformatics; K.M. Crofton, U.S. EPA / Integrated Systems Toxicology Division NIEERL; M. Linnenbrink, US EPA / ORD The limited amount of toxicity data on thousands of chemicals found in consumer products has led to the development of research endeavors such as the U.S. EPA’s Toxicity Forecaster (ToxCast). ToxCast uses high-throughput screening technology to evaluate thousands of chemicals for potential toxicity. At the end of 2013, U.S. EPA released ToxCast chemical data on almost 2,000 chemicals through the ToxCast Data Gateway (Tox21). The Tox21 Dashboard provides public access to the high-throughput screening data that can be used to inform the evaluation of the safety of chemicals. U.S. EPA recognized early in the development of ToxCast that stakeholder outreach was needed in order to translate the complex scientific information featured in the iCASS Dashboard and data, with the goal of educating the diverse user community through targeted efforts to increase data usage and analysis. Through survey feedback and the request of stakeholders, a series of tutorial videos to demonstrate how to access and use the data has been planned, and the first video of the series has been released to guide data usage. This presentation will describe the video tutorial strategy including an overview of: 1) Stakeholder outreach goals and approach; 2) Planning, production, and dissemination of tutorial videos; 3) Overview of Survey Feedback; 4) Detailed about tutorial videos easily explored. This stakeholder-outreach approach is an ongoing effort that has improved public access, understanding, and usability of the iCASS Dashboard and ToxCast data. This abstract does not necessarily reflect U.S. EPA policy.

245 FP7 SOLUTIONS project: public communication and dissemination D. López Herráez, Helmholtz centre for environmental research - UFZ / Department EffectDirected Analysis; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis; S. Consortium, SOLUTIONS SOLUTIONS is the acronym of the FP7 collaborative endeavour. Solutions for present and future emerging pollutants in land and water resources management. The project involves organizations from 14 European countries plus Australia, Brazil and China in a major effort in environmental research to ensure future ecological quality of freshwater bodies. The consortium consists of a total of 39 institutions from academia, official authorities and business, and more than 100 people working on field experiments, from physical and laboratory chemists, or from policy experts and social scientists to entrepreneurs. The core medium for raising awareness of SOLUTIONS and the dissemination of its results is an attractive Website www.solutions-project.eu conceived in close collaboration among a professional design agency and the scientific coordination team. A background section serves as front page inviting visitors to learn more into the project and its results. SOLUTIONS motivations can easily explained. Simple but captivating images, graphs and icons guide the visitors through the objectives, structure and composition of the project. A news and events section regulary updated informs about latest developments. The Web links to popular social networks such as facebook and twitter where SOLUTIONS is also present displaying expanded information. Given the international nature of SOLUTIONS, regular press releases, TV interviews or newspaper articles in participating countries and its respective languages are used to disseminate SOLUTIONS. The project’s case studies rivers (Danube, Rhine and Ebro) produce news especially interesting for local, regional and national media as well as for popular science magazines. SOLUTIONS seeks for synergies with national and European projects in related areas, e.g. SOLUTIONS puts a strong focus on thecollaboration with the FP7 projects on water resources management undermulti-stressor conditions MARS and GLOBAQUA. Part of the collaboration with its sister projects is the contribution to posts on the www.freshwaterblog.net publishing features, interviews and analyses on freshwater conservation, science and policy. This strategy amplifies the propagation of SOLUTIONS news. Internally, SOLUTIONS pursues newest ways of dialog among partners, in the form of e.g. open space discussions or Science Slam contests, which outcomes can also be used to raise general public attention on the project via the digital channels mentioned above.

246 Public communication and dissemination of structured knowledge gathered in the FP7 SOLUTIONS project through the user-friendly computer tool RiBaTox F. Sleenwaert, G. Engelen, Vlaamse Instelling voor Technologisch Onderzoek NV VITO; J. Munthe, IVL Swedish Environmental Research Institute Ltd; J. van Gils, DELTARES; R. Altenburger, UFC Centre for Environmental Research / Department of Bioanalytical Ecotoxicology; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis; D. López Herráez, Helmholtz centre for environmental research - UFZ / Department EffectDirected Analysis; S. Comero, European Commission DG Joint Research Centre; A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health The FP7 SOLUTIONS project searches for new and improved models, tools and
databases to support decisions with respect to emerging pollutants in European water resources. One important output is to provide public access by communication and dissemination of the structured knowledge gathered in SOLUTIONS through a user-friendly computer tool named RiBaTox (‘Web-based Decision Support System for River Basin Toxicants’). It will be developed for a very broad end-user community ranging from EU decision makers to the public at large. RiBaTox will be supported as ‘Decision Support System’ (DSS). It will guide the end-user to the appropriate models, tools and databases developed in the project to find solutions to stakeholder problems on assessment, prioritisation and abatement of environmental mixtures of emerging toxicants in water resources. The functionality of RiBaTox is foremost determined by the targeted end-users and the system is developed in close collaboration with potential end-users through workshops and other meetings. The ARCH project requires proactive communication and dissemination to the end-user community of preliminary results obtained in SOLUTIONS. A well-balanced communication is needed in order to translate detailed and abstract scientific results in a more comprehensible and plain language suitable for a larger user-community. The iterative ‘Evolutionary Delivery software-development Model’ (EDM) is applied to implement RiBaTox. Every EDM cycle goes through an intensive implementation phase, end-user communication, testing and assessment phase, revision of end-user requirements phase and system design phase. Currently the first EDM cycle is finalised which resulted in the formulation of the initial functional and technical specifications of RiBaTox. Valuable feedback from potential end-users was received based on a mock-up of RiBaTox distributed among them together with a questionnaire. The second EDM cycle is ongoing. We develop have been conducted a version of RiBaTox. To the effect, continuous feedback will be provided by the ‘RiBaTox End-user test Group’. This group of selected potential end-users will meet on a regular basis with a view to obtain a system that focusses maximally on typical problems encountered by end-users, that is transparent and uses a comprehensible and plain language, is user friendly and has a growth potential for future extensions.

247 Joint knowledge production to improve scientific communication and cross knowledge boundaries

A.M. Oen, Norwegian Geotechnical Inst. / Dept Environmental Engineering; A. Siiva-Tsoo / Strategy Policy Governance; G. Bredved, Norwegian Geotechnical Institute / Department of Environmental Engineering

The ARCH research project (EU-FP7, 2011-2015) aims to overcome the boundaries between the multiple scientific disciplines involved in the management of lagoon and estuary systems. This includes the challenges of communicating technical information and scientific results which are essential elements in the sustainable management of these complex systems. A central objective of the ARCH research project is to develop participative methodologies in collaboration with stakeholders to manage the multiple problems affecting lagoons in Europe. Two important components towards this goal include (i) the promotion of an integrated research approach and (ii) the employment of a true participatory process. To achieve this goal, participative methodologies have been applied at 10 case study sites in the Baltic Sea area. Workshops have been conducted which was focused on knowledge exchange to improve communication and the use of boundary objects and activities to minimize knowledge boundaries. Based on the exchange of scientific, procedural and tacit knowledge, each case study site has suggested management solutions and produced roadmaps for their implementation at the lagoon scale. Observations and experiences from the workshops will be highlighted. This includes evaluating the integrated research approach as well as drawing on comparisons between the different case study sites to explore how the context of their issues influence methods of communication and enabling stakeholders. Working in a highly interdisciplinary way emphasizes the processes of communication and interaction between stakeholders and important factors include: working towards common vocabulary and shared understanding, openness to other disciplines, acknowledging the value of other disciplines, face to face meetings, trust building and joint knowledge production. ARCH builds on the supposition that scientific limitations, including the communication of results at the end of a study, can be overcome by consciously minimizing the boundaries between multiple scientific disciplines at the forefront of investigations and that scientific knowledge can be improved by addressing social systems.

248 The scientific think tank project Baltic Eye- Science and communication to inform and support decision making for the Baltic Sea environment

K. Broeg, H. Hamrén, H. Markstedt, Stockholm University / Baltic Sea Centre (Torekällberget); E. Spiewok, a. closed bottom sea, has been declined by pollution and unsustainable fishing for decades. Even though the Baltic Sea countries, the EU, HELCOM, and ICES have recommended and taken numerous measures to reduce contaminant and nutrient inputs and increase fish stocks, the environment has not recovered. There is need to disseminate knowledge on the Baltic Sea ecosystem and the impact of different stressors, alone and in concert, to provide decision support for policy making. For that purpose, Stockholm University and the Baltic-Sea2020 foundation initiated the scientific think tank Baltic Eye with a core team of seven researchers and three communicators, covering the main impact areas, eutrophication, fisheries, contaminants and agriculture. Baltic Eye seeks society’s need for knowledge as well as the relevant communications-, policy-, and decision landscapes. With respect to contaminants, Baltic Eye acts as an arena for synthesizing existing and initiating new scientific knowledge, building-up networks, and communicating new information on sources, fate and impacts as well as suggesting measures. Communication mainly targets decision makers at regional, national, and European levels. As an example, the Baltic Eye project on microplastics in the Baltic Sea and its communication strategy is presented.

Soil and water pollutants’ assessment, monitoring and remediation (I)

249 Chemical Speciation of Organotin Compounds in Seawater from Cape Town Harbour, South Africa; Seasonal variations and their Toxicity Effects

O.S. Fatoki, Cape Peninsula University of Technology / Chemistry

Water samples were collected in twelve locations from Cape Town harbour in order to assess their tributyltin and triphenyltin contamination. Moreover, seasonal variation and their toxicological effects were examined using biomarkers. The annual concentration ranged from 0.067 ± 0.01 to 111.290 ± 32.20 x 10⁻³ µg/l for TBT while that of TPT ranges between 23008.0±0.3 x 10⁻³ µg/l respectively between locations. The seasonal variation of TBT was also investigated; significant variation of P < 0.05 was found after the statistical analysis. Seasonal variation in TBT and TPT concentrations with a higher level in summer than in winter and spring has been observed. Apparently, the observed high or low value recorded for organotin compounds in Cape Town harbour could be as result of increase or decrease in the traffic of ships and boats. More over dilution due to increase in water volume results in decrease in concentration of TBT. High value observed for TBT in summer could be associated to steady flow of water, while in spring, the ocean tide effect may suggest reason why we have more TBT in spring or low but this also depends on the direction of the tide. The toxicity effects of tributyltin were investigated using responses of lysosomal membranes of hemocytes of the mussel, Mytilus galloprovincialis, as a biomarker of stress due to exposure to tributyltin. Apparently, from this study, for both exposures group NRR time became progressively shorter as TBT concentration increasing with time. This study has revealed that the two contributing factors influencing lysosomal responses are exposure concentration and exposure time of TBT. The NRR time assay could be considered as a useful cellular biomarker of stress due to tributyltin exposure.

<strong>250 Civil Aviation Initiative on Climate Change Impact Assessment on Ground Water Resources in Libya - D.P. Bindra, Civil Aviation / UNCSD Rio Focal Point</strong>

This paper is designed to present the likely impact of climate change mainly due to increased human activities like growing aviation industry & petroleum E&P on groundwater resources in general and Libya in particular. State of the art reviews on recent research studies, and methodology to assess the impact of climate change on groundwater resources shows that climate change poses uncertainties to the supply and demand of water, especially on Libya coastal areas where most of the agriculture, domestic and industrial activities are concentrated. Based on several research studies to combat GHG like Green Sky Initiative of Civil Aviation College Sbia in Libya it demonstrates that how policy and decision making process using best practices for monitoring, analyzing and forecasting variation of climate is a way forward to cope with the impact of sea level rise, and combat some water supplies in vulnerable areas that are becoming unusable due to the penetration of salt water into coastal aquifers. Finally, interesting results from a number of initiatives like sustainable aviation using green sky initiative in the form of posters by students are reviewed to demonstrate that how better understanding of climate and climate change forecasting helps in devising appropriate adaptation strategies due to the impact of climate change.

251 Evaluation of the potential of the Microbial Assay for Risk Assessment of herbicides

P.B. Bi Asanging Pai, Ecotoxicologist Senior Lecturer / animal biology; M. Pomoane, N. Norbert, P.F. Tankie, University of Dschang / Department of Animal Biology, Cameroon

Herbicides, especially insecticides and fungicides, are more heavily applied to tropical cash crops, e.g. banana, coffee, cotton, and vegetables, than to crops in temperate regions. A big data gap exists in Africa as far as levels of pesticides and toxic effects in the environment are concerned. The microbial Assay for Risk Assessment (MARA) is a multi-species test with 11 taxonomically diverse microbial strains freeze-dried in a 96-well microplate used to assess the ecotoxicity of chemicals and environmental samples. However there is little published data on the sensitivity of MARA test to pesticides, particularly herbicides. This research set out to investigate how representative MARA bioassay responses are of effects of herbicides in the aquatic ecosystem with the aim of establishing how useful it can be.
in filling in the data gap. In this research we compared the sensitivity of MARA to a fish bioassay using two commonly used herbicide formulations: Round up (a.i. glyphosate) and herbextra (a.i. 2,4-D). The mean MARA MTC for glyphosate was 101.82 mg/l with a range of MTCs from 6.85 to 172 mg/l for the various microorganisms. In the case of 2,4-D MTC values obtained ranged from 74.67 mg/l to 13,333.33 mg/l with a mean value of 2855.88 mg/l. On the other hand, 96% Ethylene glycol was used as standard control (R²=0.9981). The activated charcoal from Uncaria tomentosa fingerslingers were 11.62 (10.5-11.64) μg/L, 222.28 (209.86-228.11) μg/L and 23.07 (21.95-24.35) μg/L respectively for glyphosate and 2,4-D. The two herbicides were highly toxic to the MARA microorganisms at environmentally relevant concentrations with glyphosate being at least 20 times more toxic than 2,4-D. However, when compared to effects on fish, the MARA microorganisms were far more tolerant to the herbicides, showing that MARA microorganisms responses do not reflect responses from aquatic organisms from all trophic levels. This implies that while the MARA test may have a great potential for assessing eco-toxicological effects of pesticides and other environmental pollutants on the microbial community, for better protection of aquatic ecosystems other bioassays are necessary to be included in the test battery, particularly algal or plant bioassays.

252 Monitoring of fluoroquinolone antibiotics in piggy wastewater and their mobility in solid materials after the application of the slurry as fertilizer V.D. Latskas, Department of Civil and Environmental Engineering University of Cyprus; A.K. Parpounas, NIREAS-IWRC / Civil and Environmental Engineering University of Cyprus; C. Mitas, C. Xie, CIP, Xinxiang 653000, China; and NIREASInternational Water Research Center; D.F. Kassinos, NIREAS-IWRC / Civil and Environmental Engineering Enrofloxacin (ENR) is regularly administered, in a global scale, in concentrated animal feeding operations (CAFOs) for therapeutic and prophylactic purposes. As a result, the repeated application of contaminated animal wastewater as fertilizer could enrich the soil with ENR and its metabolite, ciprofloxacin (CIP). Nevertheless, data for their presence and concentrations in animal wastewater are limited, as well as data for their mobility in soils. In this study, piglets’ urine and faeces mixture samples as well as piggy wastewater samples were collected from several points (e.g. pregnant and nursery facilities, weaning facilities, primary wastewater collection tank) in a CAFO located in Cyprus to monitor ENR and CIP. The present study describes the development and application of a fast and accurate analytical method for the investigation of the occurrence of fluoroquinolones (FQs) in piggy wastewater, using off-line solid phase extraction (SPE) followed by UPLC-MS/MS. The extraction procedure based on the formation of FQs-MgSO4 complexes allowed the desorption and extraction of both antibiotics from wastewater, which was not feasible by using conventional organic solvents. The recoveries for both antibiotics from wastewater were at least 85% and limit quantification was lower than 1 μg L-1. In addition, batch equilibrium studies were performed to examine the adsorption of ENR and CIP in a clay soil, in quartz sand and in solid material from swine slurry. The results showed that ENR concentrations in the collected wastewater samples ranged from 7 to 40 μg L-1 while the concentrations of CIP were 130 to 360 μg L-1 in the various wastewater samples. The adsorption experiments showed that clay soils as well as the solid material from swine slurry have a great potential to immobilize these antibiotics. The concentration specific adsorption coefficient (Kads) values ranged from 412 to 5075 L kg-1 for ENR and 155 - 3365 L kg-1 for CIP, respectively. The swine slurry solid material has shown the highest adsorption capacity for both the antibiotics, followed by the clay soil and the sand, in the order given. Our results indicate the presence of ENR and CIP in the swine wastewater, even when the antibiotics are administered at low doses. The soil properties (e.g. clay, silt, sand, organic matter content) determine its ability to immobilize ENR and CIP antibiotics and consequently the capacity of the terrestrial ecosystem to assimilate them.

253 Removal of PFOS and PFOA from aqueous solutions using activated charcoal of Vitis vinifera leaf litter B.O. Opeolu, Cape Peninsula University of Technology / Faculty of Applied Sciences; B.O. Fagbeyigbo, Cape Peninsula University of Technology / Environmental Health; M.O. Ncube, Computational studies; O.S. Fatokun, B.J. XIMBA, O.S. Olutunji, Cape Peninsula University of Technology / Chemistry In recent times, quality of freshwater resources have been deteriorating due to pollution from diverse sources. In Africa, emerging contaminants such as PFOS and PFOA are becoming prevalent in environmental samples. Negative effects of compounds such as PFOS and PFOA on man and environment have been reported elsewhere. The need for thorough studies into the development of processes that cannot be over-emphasized given the current drive of developing nations’ for industrialization. It is therefore important to proactively consider ways of protecting ecosystems from pollution. In this study, activated charcoal sourced from waste leave biomass of Vitis vinifera were investigated in order to evaluate their efficiencies as adsorbents for removal of PFCs from water. V. vinifera leave biomass debris was collected from wine farmland in Stellenbosch, Western Cape South Africa. The leave biomass was air dried, pulverized, carbonized under N2 gas at 550°C and treated with 1M phosphoric acid and 1M potassium hydroxide. Batch experiments were carried out to obtain data for adsorption equilbrium isotherms and fitted into kinetic models using simulated wastewaters containing perfluorinated compounds. The effects of concentration, pH and adsorbent dosage on the removal efficiency of the PFCs were observed. Data fitted well into the Freundlich isotherm model. There was increasing removal of the PFCs with increasing concentration with an optimum value of 0.75g/mL. Adsorption of PFOS and PFOA were best described by Freundlich model for acid activated charcoal with R2 values of 0.9957 and 0.9992 respectively. Corresponding values for KOH activated charcoal were 0.9951 and 0.9901 respectively. This suggests multilayered adsorption. Values of n evaluated from the slope of the plots are indicative of favourable adsorption. Increased adsorbent dosage also enhanced removal of PFOS and PFOA. Adsorption kinetics was best fitted into pseudo second order kinetics with R2 values 0.9911 and 0.9858 for PFOS and PFOA respectively with KOH activated charcoal. Corresponding values for H2O2 activated charcoal were 0.9234 and 0.9477. Maximum adsorption capacities qmax are 140 mg/g and 107 mg/g for PFOS and PFOA respectively.

254 Life in a polluted environment: an interdisciplinary approach on the effects of contaminants in the brown shrimp, Crangon crangon D. Mondal, University of Salford / Environment and Life Sciences; C. Benvenuto, University of Salford / Environment and Life Sciences Introduction: The brown shrimp Crangon crangon L. (Decapoda, Caridea) is distributed all along the European coasts and uses estuarine areas, potentially impacted by human pollution as nursery ground. Like many other crustaceans, these shrimp play a key role in the ecosystem and are economically important as key components of food webs. Shrimp reared in low biomass concentration in the protein in our diet. We aim to use C. crangon as bioindicator and biomonitor of environmental quality, in particular to detect the effects of environmental pollutants on morphological, physiological, behavioural and life-history adaptations, as well as on its fascinating mating strategies. Our sampling site is a unique semi- isolated pool near the Upper Mersey River, Widnes, UK. Hence, collecting the shrimp from a confined polluted environment from a historically contaminated area makes project significantly different compared to studies done only in the laboratory by dosing the animals. Methods: Samples were collected for shrimp, sediment and water from the sampling pool along with three other locations (Mersey River, estuaries at Milford Haven, Wales, and Praia de Faro, Portugal) for comparative studies. Analyses of heavy metals and PCBs in the whole body of shrimp along with the sediments and water were performed following established protocols. Molecular analyses to assess genetic variation and microbiota were piloted. Behavioural studies like chromatophore experiments and sex changing experiments are in progress. Histopathological and cytotoxicological tests are being conducted. Results: Heavy metal sediment concentrations were up to two orders of magnitude higher in the sampling pool compared to the three other locations (p<0.05), while the concentrations in the water were comparable. Keeping in mind that these animals are benthic, surprisingly, the xenobiotics like arsenic and chromium were lower in the shrimp collected from the sampling pool compared to the other areas (but not significantly, p>0.05). PCB concentrations were higher in the shrimp collected from the sampling pool. Conclusion: The presence of high concentrations of PCBs in the sampling pool is likely to cause harm to the shrimp collected from the sampling pool leaves us with questions on adaption strategies of the shrimp dwelling under this special environmental condition and we are in the process of addressing these based on the on-going experiments.

Epigenetic effects of chemicals in ecological and human risk assessment: challenges and perspectives

255 A new paradigm in chemical hazard assessment: Chemicals do not induce genomic mutations but are epigenetic agents! J. Trosko, Michigan State University / Department of Pediatrics/Human Development The foundation of a rigorous risk assessment is a scientifically sound understanding of the mechanisms by which chemicals cause various health effects. There are three mechanisms of toxicities, “mutagenesis”; “cytotoxicity” and altered expression of gene expression at the transcription, translation and posttranslational level of “epigenetic toxicity”. As more and more chemical toxicities has been that of “genotoxicity”, a challenge will be made that chemical toxicities toxins/toxicants do not cause genomic DNA damage or mutations that contribute to birth defects, to the multi-stage, multi-mechanism process of carcinogenesis, immune-toxicity, reproductive – or neuro-toxicities. Rather, the primary mechanism associated with natural and synthetic chemicals, to which multi-cellular organisms are normally exposed, are effects on the health and survival of exposed organisms, during food processing, and supplements, is that of the inappropriate modulation of extra-, intra- and inter-cellular communication. This delicate and integrative series of communication mechanisms is the basis for homeostatic control of cell proliferation, differentiation, apoptosis and senescence of organ-specific adult stem cells. While mutations do contribute to germ-line and somatic diseases, such as atheroscleroma (‘errors in DNA repair’) and Bloom’s syndromes (‘errors in DNA replication’), non-mutagenic chemicals, such as DDT, phorbol esters, PBB’s, PCB’s, TCD’s, phenobarbital, phthalates, asbesson, estrone, poly aromatic hydrocarbons, thalidomide, asbestos, aflatoxin, etc., all of which can
induce ROS’s and induce oxidative stress, trigger various intra-cellular signaling to (a) modulate gap junctional or extra-cellular signaling to disrupt homeostasis in a
multi-cellular organism. This class of epigenetic agents is characterized by (a) having
species, gender, developmental stage specificities; (b) having threshold levels of
action; (c) exhibit toxic consequences during “critical windows” during development
or during regular & chronic exposures in the adult; and (d) must be present in the absence of antioxidant agents. Understanding the many specific mechanisms by which these epigenetic chemicals can modulate the quality and quantity of stem cells is critical if we hope to have rigorous risk assessment.
Disruption of the quantity of organ-specific adult stem cells during fetal/neonatal
development is probably the mechanism behind the “Barker Hypothesis”.

256 Epigenetic mechanisms in invertebrates and their potential role in responses to
environmental pollutant exposure
D. Spurgeon, Centre for Ecology & Hydrology

257 Use of differentiating human stem cell cultures to define epigenetic
mechanisms of developmental toxicity
M. Leist, University of Konstanz / Faculty of Mathematics and Sciences

258 Interactive discussion: addressing approaches and perspectives regarding the
risk assessment of epigenetic effects
H. Gaugtisch, Umweltbundesamt (Environment Agency Austria) / Landuse
Biosafety

259 Toxicokinetics and epigenetics: implications for human and ecological risk
assessment
J. Dorne, Scientific strategy

260 Potential role of information on epigenetic mechanisms in regulatory risk
assessment of chemicals
H. Hunskem, European Chemicals Agency

Interpreting Biological Effects of Metals and Their Mixtures (II)

261 Evaluation of immune and acclimation capacities of the three-spined
stickleback (Gasterosteus aculeatus) in polymeric contamination context.
A. Le Guernic, IRSNPRP-ENVLECO / BouchesduRhône; w. sanchez, FCS
Rovaiian / General Direction; S. Betoulle, Université de Reims; B. Gagnaire,
In an ecosystem, organisms are exposed to multiple and different stressors. These
stressors can act individually or in combination. Studies start to take into account
these multiple stressors and environmental variations for ecotoxicologic risk
assessment. The aim of this study was to evaluate the impact of polymeric
contamination on acclimation capacities of a fish, the three-spined stickleback, after
a stress challenge (chemical and biological). Chemical stress was defined by the
polymeric contamination linked to former uranium mines. Moreover uranium
other trace metals, naturally occurring (Fe, Mn) or introduced by man to precipitate
uranium and its decay products (Ba, Al), increased the metal contamination of these
sites. Three-spined sticklebacks were caged in three ponds in Haute-Vienne
department (France). A pond was chosen for its proximity to former uranium mines
(Pontabrier pond) and two other ponds were selected outside the influence of these
tailings (Malessa and Jonchere Saint-Maurice). Two in situ experiments were
conducted to know if seasonal (April and September-December) and duration of
metallic exposure impacted stress responses of sticklebacks to LPS. Fourteen days
after metallic exposure (chemical stress) for the first experiment and after 76 days
for the second, fish were subjected to an injection of lipopolysaccharide (LPS)
corresponding to a biological stress. Finally, four days after injections, sticklebacks
were euthanized for analysis of immunotoxicity, oxidative stress and genotoxicity.
Although the concentrations of iron, aluminum and manganese were important for
each pond, the highest metal concentrations were found in the pond subjected to
mine tailings (Pontabrier), especially for uranium. Chemical stress impacted negatively
the major biological functions analysed in stickleback, as well as the
biological stress. Chemical stress reduced responses of sticklebacks to biological
stress by decreasing phagocytic and antioxidant responses to LPS. Nevertheless,
epigenetic effects could be observed in some biomarkers analysed. Results of the
second experiment will help to know if seasonal or exposure period had an impact on
responses recorded.

262 Mixture toxicity for metals in the environment: potential for refinement of the
concentration addition approach.
K. Oorts, ARCHE C. Moens, Katholieke Universiteit Leuven / Division Soil and
Water Management; F. Verdonck, ARCHE
The assessment of mixture toxicity of naturally occurring elements, such as metals,
in the environment based on information on the individual components is
challenging. Several important aspects, such as natural background contamination,
ecotoxicity, differences in bioavailability and bioaccumulation can significantly affect
the outcome of the assessments. For several metals, the predicted no-effect concentrations (PNEC) are
close to their natural background concentration, and adding the predicted potential
effect of several metals may lead to over-protective results and even identification of
risks in the natural environment. In the absence of adequate mode of action information
for all compounds and concentrations, the concentration addition (CA) method is the
default, conservative concept for estimating toxicity from chemical mixtures.
Furthermore, concentration addition can be applied based on the available toxicity
data (EC10, EC50, …) for the individual components and additional testing is
therefore minimized. Recently, a tiered approach for mixture toxicity assessment
of biocides was published (ECHA, 2014). In this document, some refinements of the
standard CA method are presented. These approaches were applied for prediction of
mixture toxicity for metals in water and soil. The results demonstrate limited
potential for improvement of the standard concentration addition approach for a
multi-metal mixture, even when the assessment is based on species-level. Correct
prediction of potential effects of the individual components at low exposure (i.e.
below PNEC) is critical for prediction of mixture toxicity. However, the standard
extrapolation of effects and risks between the toxicity threshold (e.g. PNEC) and
absence of a substance in the environment generally yields an over-estimation of
potential risks at low exposure concentrations. When sufficient data are available
for the derivation of a species sensitivity distribution for the individual components, it
is therefore proposed to use the potentially affected fraction at the exposure
concentration of the individual metals as a basis for the summation in the
concentration addition approach.

263 Incorporating bioavailability into toxicity assessment of Cu-Ni, Cu-Cd, and
Ni-Cd mixtures with the extended biotic ligand model and the WHAM-Fiox
approach
H. Qig, Leiden University; M.G. Vijver, CML Leiden University; E. He, VU
University Amsterdam / Department of Ecological Science; W.J. Peijnenburg,
RIVM / Center for Safety of Substances and Products
Seedlings of lettuce Lactuca sativa were exposed to metal mixtures (Cu-Ni, Cu-Cd,
and Ni-Cd) contained in hydroponic solutions for 4 d and inhibition to root
elongation was the primary endpoint used to quantify toxic response. The general
objective is to incorporate bioavailability in the assessment of mixture
toxicity using the the extended biotic ligand model and the WHAM-Fiox approach.
By adopting an f_{act} value, the extended biotic ligand model succeeded to predict
toxicity of Cu-Ni, Cu-Cd, and Ni-Cd mixtures to L. sativa, with more than 82% of
toxicity variations explained. The validity of the model suggests that competition is
the dominant mechanism accounting for interactions between mixture components.
There were no significant differences in the values of f_{act} for the three mixture
combinations, showing the possibility of extrapolating these f_{act} values to other
binary metal combinations. The WHAM-Fiox approach, postulating that
competitive chemical reactions at one or more biotic ligands of the organism can be
represented by the competitive binding to particulate hemic acid, had a similar level
of prediction in estimating mixture toxicity while requiring fewer parameters than the
BLM-fiox model. External validation of the WHAM-Fiox approach using literature
data showed its applicability for other species and other mixtures.
Generally, the WHAM-Fiox model works when the BLM also works. Based on our
finding, the WHAM-Fiox is recommended as an effective approach to incorporate
bioavailability in quantifying mixture toxicity. Theoretically, the WHAM-Fiox
approach can be applied to predict toxicity of binary, ternary, and quaternary (or
even more) metal mixtures, provided that competition at the biotic ligand can be
simulated on the basis of assumptions of competition at abiotic ligands.

264 Four methods to estimate potential risks of metal mixtures: a case study for
Flemish waterways
T. Van Regenmortel, Laboratory of Environmental Toxicology and Aquatic
Ecology GHEnToxLab Unit; C. Janssen, University of Ghent / Laboratory of
Environmental Toxicology and Aquatic Ecology GHEnToxLab unit; K.A. De
Qiu, RIVM / Centre for Safety of Substances and Products
In the EU and worldwide, the assessment and management of potential risks posed
by chemicals is almost exclusively based on evaluations of individual substances.
This study evaluates potential risks of metals occurring in four-metal (Cu, Zn, Ni,
Cd) mixtures when each metal is present in the environment at its biotic ligand
model (BLM) based, bioavailability-corrected environmental concentration. Four
different methods - combining the concentration addition (CA) model or the
Independent Action (IA) model with Species Sensitivity Distributions (SSD) in
four different ways - were used, critically evaluated, and compared. For all four
metals, high-quality chronic EC10s, EC50s and the corresponding water
characteristics were compiled in a database. Using biotic ligand modelling, all
EC10 values were normalized to the physico-chemistry of 169 Flemish water bodies. The information of these water bodies was collected from databases of the Flemish Environmental Agency. Using CA and IA, the fraction of potentially affected species per water body and the percentage of water bodies affected was evaluated. A water was considered 'potentially affected' when the Potentially Affected Fraction (PAF) of species was higher than 5%. Results indicate that the most simple of the four methods, i.e. the CA-SSD method, predicts the highest percentage of water bodies affected (34%), and is therefore the most conservative method. However, of these 34% waters that are potentially affected, only 28% are affected by the mixture itself, and not already by the individual metals. Potential risks caused by metal mixtures seem to be dominated by risks caused by individual high Zn concentrations (58%). When examining potentially affected waters caused by specific mixture effects, Zn and Cd contributed the most to the mixture effect (37 and 30%, respectively). This study demonstrates that using CA and IA for the risk assessment of metals implies that environmental risk will be predicted for a substantial amount of water bodies in the Flemish waterways. However, mixture specific risks, in contrast to mixture risks caused by individual substances alone, are limited. The outcome of the four methods used here show small differences. However, the validity of these predictions needs to be further evaluated using dedicated laboratory and field studies.

265 New advanced methods for modelling toxicity of metal mixtures to plants

Y.T. Le, Radboud University Nijmegen / Environmental Science

Mechanisms of ion-sorption surface interactions simulated to explain the variations in metal toxicity. Besides environmental chemistry, biological responses of plants exposed to metal mixtures are affected by interactions between the metals and interactions of the metals with the plants. These interactions might be taken into account in estimating metal toxicity to plants with three methods applied recently. The first method is based on the assumption of the Biotic Ligand Model (BLM) that metal accumulation in roots is controlled by competition between metal ions for binding sites at the ligands, which determines toxic effects (BLM-based). The second assumes that metal sorption to the plasma membrane, which is determined by the electrical potential at the membrane, is directly related to metal toxicity (electrostatic model). In the last approach, metal accumulation in roots, which links to biological responses, is simulated by metal binding to humic acid in geochemical speciation models (WHAM-based). These models can be generalised as using metal affinity for binding sites on sorption surfaces (i.e., biotic ligands, plasma membrane surface, and humic acid) as a unifying factor of metal toxicity. The potential of the three models for estimating the toxicity of Cu–Zn2+ and Cu2+–Ag+ mixtures to lettuce Lactuca sativa was evaluated with the same concept, i.e., toxic units. The potential for simulating ion-ion interactions was the decisive factor for predicting toxicity of metal mixtures with these models. The electrostatic model was found least effective while the WHAM-based model was only slightly better than the BLM-based model in predicting the growth of lettuce roots exposed to the mixtures. These results are potentially related to the negligible variations in the electrical potential at the plasma membrane surface and the differences between roots (i.e., toxic units). These potential for simulating ion-ion interactions was the decisive factor for predicting toxicity of metal mixtures with these models. Both electrostatic interactions and metal/site-specific binding at sorption surface are included in the WHAM-based model. The former is included in the BLM-based model while the latter is not completely addressed in the electrostatic model.

266 Mixture toxicity of Cu, Ni, Cd and Zn to barley can conservatively be predicted by the concentration-addition model

L. Versieren, EES; K.A. De Schampheelaere, Ghenent University (UGent) / Laboratory for Environmental Toxicology and Aquatic Ecology GherToxLab unit; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research Department of Biology; E.E. Smolders, Katholieke Universiteit Leuven / Division Soil and Water Management.

Metal contamination is most often a mixture of different metals and these multi-component mixtures could produce significant mixture effects. There is a need to understand how metals act together in producing combination effects, especially at low effect concentrations of the individual metals. The present study aimed to investigate mixture effects of Cu, Ni, Cd and Zn at low effect concentrations (Cu, Ni and Zn to barley root elongation in nutrient solutions at metal concentrations individually causing low level effects). The objective was to test whether the CA model, suggesting different modes of actions of the metals in the mixtures. The CA model, however, is generally more conservative than the IA model and can be used as a safe model to predict metal mixture toxicity to barley. Mixture treatments with binary and ternary combinations of Cu, Ni and Cd generally produce small synergistic or additive interactions, whereas mixtures with elevated Zn concentrations lead to additive and antagonistic interactions, mainly Cd + Zn mixtures. Potentially antagonistic interactions.

Plastics in the Aquatic Environment: Mechanisms and Implications (II)

267 Ingestion of microplastics by freshwater invertebrates

C. Schöger, Aquatic Ecotoxicology; N. Brennhoft, Federal Institute of Hydrology / Biochemistry and Ecotoxicology; G. Reifferscheid, Biochemistry and Ecotoxicology; M. Wagner, Goethe University Frankfurt / Aquatic Ecotoxicology Plastic items are accumulating in aquatic systems worldwide. Under environmental conditions, plastic materials degrade to so-called microplastics (MP, < 5 mm). Although laboratory studies demonstrate an uptake of MPs by a broad range of marine organisms, data on freshwater organisms are lacking. To evaluate the uptake and fate of microplastics in freshwater invertebrates and to establish a basis for future ecotoxicological studies, we conducted ingestion studies with MPs and freshwater invertebrates. Daphnia magna, Gammarus pulex, Chironomus riparius and Physella acuta were exposed to different concentrations (3-3000 particles mL−1) of fluorescent polystyrene particles (10 and 90 µm in diameter) in short-term experiments. To test the influence of additional natural particles, we included food or sediment in our testdesign. Our results clearly demonstrate that freshwater invertebrates ingest and accumulate high amounts of MPs. In the presence of natural particles (food/sediment), the overall ingestion as well as the ingestion rates decreased. We conclude that MP ingestion is determined by the size of the particles as well as the feeding behavior, the stage of development and the size of the organisms.

268 Potential effects of microplastics on limnic zooplankton

S. Rehse, Leibniz-Institute of Freshwater Ecology and Inland Fisheries / Centre for BioNano Interaction. Nanoplastics debris represent an emerging concern for marine ecosystems. Nevertheless, their fate and effects of microplastics in freshwater systems. Our aim is to systematically test the uptake and effects of microplastics on freshwater organisms on different levels of the food chain. In a first step, we exclude chemical effects, e.g. by additives or attached pollutants, by using raw microplastic particles. As a model organism for limnic zooplankton we exposed Daphnia magna to different types of microplastics (polyethylene, 1 and 100 µm particles). Before exposing neonates of Daphnia magna to the water, we characterized. Both particle sizes (1 and 100 µm) tended to float at the water surface. However, the majority of 1 µm particles remained in the water column after vigorously shaking. In contrast, the 100 µm particles were floating back to the surface immediately after mixing. This led to different exposure scenarios for the daphnids. After exposure, the 1 µm particles were ingested by the daphnids and accumulated in the carapax. Both uptake and accumulation of 1 µm particles increased with higher concentrations. The 100 µm particles, in contrast, were neither ingested nor accumulated at the carapax. We conclude that different sizes of microplastics (1) lead to different behaviour in water followed by different exposure scenarios for organisms and (2) induce different effects, e.g. like physical effects by accumulation on the surface of the carapax of daphnids. Attached particles might cause problem by melting and lead to different effects e.g. in growth. Our results thus provide a first basis to systematically identify parameters of microplastics that determine adverse impacts on freshwater organisms on different levels of the food chain.

269 Nanoplastics impacts on marine organisms: accumulation and toxicity of polystyrene nanoparticles in three target species

e. bergami, University of Siena / Physical Earth and Environmental Sciences; C. Dellà, Torre, University of Siena / Department of Physical Earth and Environmental Sciences; C. Ciacci, University of Urbino; A. Salvati, University College Dublin / Centre for BioNano Interactions School of Chemistry and Chemical Biology; e. bocci, University of Siena; M. Vannuccini, University of Siena / Physical earth and environmental science; e. faleri, University of Siena / Department of Life Sciences; p. ciurio, Anton Dohrn Zoological Station; K.A. Dawson, University College Dublin / Centre for BioNano Interaction School of Chemistry and Chemical Biology; L. Canesi, university of genoa / DISTAV; I. Corsi, University of Siena / Department of Physical Earth and Environmental Sciences Nanoplastics debris represents a growing concern for marine ecosystems. Nevertheless, their fate and impact on marine biota is almost unknown. Polystyrene nanoparticles (PS NPs) can be considered as model for studying both fate and toxicity of nanoplastics in marine organisms. Marine invertebrates are considered primary biological targets of polymeric NPs, being exposed both in the water column (phyto- and zooplankton) including bivalves and in sediments (grazers and bottom feeders). For this purpose, the present study evaluated the accumulation and toxicity of 40 nm anionic carboxylated (PS-COOH) and 50 nm cationic amine (PS-NH2) NPs in three target marine species: brine shrimp Artemia salina larva, mussel Mytilus galloprovincialis tissues and sea urchin Paracentrotus lividus early development. Secondary characterisation of PS NPs in natural sea water (NSW) (0.45 µm filtered, T 18°C, salinity 39‰, pH 8.3, conductivity 6 m/S) was
performed using DLS analysis. PS-COOH resulted massively sequestered inside the digestive tract of sea urchin embryos at 48 hours post-fertilization and brine shrimp after 48 h exposure. PS-COOH were exchanged between gills and circulatory system after 2 h of exposure and accumulate in lysosome of mussel’s haemocytes. No relevant toxicity was observed for PS-COOH in all three target species. PS-NH₂ resulted less clear disposed both in brine shrimp larvae and sea urchin embryos after 24 h of exposure, consistent with previous research. In mussels’ haemocytes, exposure to PS-NH₂ caused an increase in extracellular ROS and NO production and also dose-dependent modulation in phagocytosis and lysozyme activity was observed. The clear differences in accumulation and toxicity of PS-COOH and PS-NH₂ might be related to their surface charge and aggregation in sea water. Our findings suggest that these three species are vulnerable to PS NPs and further research is strongly encouraged on specific pathways of toxicity in marine organisms.

270 Formation of microplastics and bioaccumulation of toxic additives by lugworm inhabiting in expanded polystyrene marine debris

E. Besseling, Korea Institute of Ocean Science and Technology / oil and POPs research group OPRG; S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; G. Han, Oil and POPs research group; M. Rani, Korea Institute of Ocean Science and Technology; Y. Song, Oil and POPs research group; W. Shim, Korea Institute of Ocean Science and Technology / Oil and POPs research group

Dugongs (less than 5 mm in size, numbers of microplastic has increased in oceans worldwide. Fragmentation of plastics in the environment was expected mainly at beaches where photo-degradation and abrasions take place through wave actions. However, bioactivities of organisms in floating plastics could also contribute to the formation of microplastics. Large amounts of expanded polystyrene (EPS) buoys have been used in aquaculture activities in South Korea. Lugworm (Marphysa sorbents) is a marine organism generally found in mud and rock. Interestingly, many burrows of this species were found inside EPS buoy while searching organisms inhabiting on EPS buoy. In our previous study, considerable amounts of HBCDs in EPS buoys were detected. HBCD has been categorized as a persistent organic pollutant and is now listed in Stockholm convention in 2013. We try to determine (1) whether microplastics are created by burrowing of lugworm, and (2) lugworm can accumulate additive HBCDs from EPS buoy. EPS buoys were collected from the coasts of Geoje in South Korea. After collection of EPS buoys, lugworms and EPS particle inside burrows were sampled. Sampled lugworms were kept separately for 3 days for cleaning of gut and collection of excretions. EPS particles in lugworm’s excretions identified using FTIR were roughly globular in shape. The size of EPS particles were varying according to the size of lugworm. The maximum length (length means the longest axis of EPS particles) and median length were 3.79 mm and 1.26±0.83 mm for large size lugworm (weight: 6.38 g, length: 26 cm), and 1.97 mm and 0.84±0.42 mm from small size lugworm (weight: 3.27 g, length: 14 cm), respectively, displaying dependency on mouth size of lugworm. The mean number and weight of EPS particles per excretion from lugworm (n=10) were 132±121 and 2.25±3.17 mg, respectively. HBCDs concentration was found according to their substrate types, indicating that lugworms (n=10) were 132±121 and 2.25±3.17 mg,

271 Chemical Pollutants Adsorbed to Microplastics Bioaccumulates in Exposed Fish

P. Wardrop, RMIT University / School of Applied Science; J. Shimeta, P. Morrison, RMIT University; A.F. Miranda, School of Applied Sciences / School of Applied Sciences; M. Tang, D. Nugeoda, B. Clarke, RMIT University / School of Applied Sciences

The contamination of the natural environment with plastic debris is an increasingly prioritised concern among regulators, scientists and citizens. Aside from photo-degradation and abrasions take place through wave actions, negative impacts on natural aesthetics, plastic debris causes a range of ecological harm from entanglement to starvation when mistakenly ingested by animals Plastic adsorbs and concentrates pollution from the surrounding environment but the role that plastic debris plays in the movement of chemical pollution and contamination of food chains is largely unknown. Recent studies show that plastics facilitate the bioaccumulation of pollutants to a variety of exposed organisms. Here we show for the first time that fish fed small microplastic particles (s-MPPS), isolated from a commercial facial cleanser and contaminated with the persistent organic pollutants polychlorinated diphenyl ethers (PBDEs), bioaccumulated PBDEs in their tissues.

272 Relative importance of PCB uptake from sediment and microplastic by the lugworm Arenicola marina (L.)


Microplastics have been hypothesized to be a vector for POP transport between organisms and the aquatic environment. Both model and experimental work previously indicated that microplastics might increase body concentrations by up to a factor of two. To enhance comparability with environmental scenarios and validate model simulations, however, additional studies are needed. To date, most plastic exposure studies that involve contaminants use chemicals spiked to the plastics only, whereas in the natural environment, ingestion of regular food items may represent an equally or more important pathway of uptake. Therefore, the relative role of plastic ingestion as an exposure pathway to marine organisms is unknown. Here we use a novel set-up that allows to distinguish between plastic and regular food-mediated transfer of PCBs, by applying matched PCB congener couples in plastic and food respectively. In this set-up, the marine lugworm Arenicola marina was exposed to 10-180 polyethylene microplastic and PCBS for 28 days. The endpoints mortality, feeding behaviour and weight as well as PCB concentrations in tissue and sediment were analysed. In this way, more insight was obtained in the effect that microplastics have on the distribution of POPs among compartments.

Latest advances in passive sampling and dosing (II)

273 Calibration of Polydimethylsiloxane and Polyurethane Foam Passive Air Samplers for Measuring Indoor SVOCs

J.O. Okeme, University of Toronto / Physical and Environmental Sciences; A. Sanni, University of Toronto / Department of Physical and Environmental Sciences; C. Yang, University of Toronto / Department Earth Science; L.M. Jantunen, Environment Canada; M.L. Diamond, University of Toronto / Department of Earth Sciences

Passive air sampling has become a popular and efficient alternative to active air sampling for semi-volatile organic compounds (SVOCs). Polyurethane (PUF), polyurethane and benzene copolymer (XAD), and polymer coated glass (PG) passive air samplers (PASs) are well characterized for measuring SVOCs. These methods work well outdoors but are not well characterized for the indoor environment. We introduce a new passive air sampler (PAS) consisting of polydimethylsiloxane (PDMS), PDMS has been used for the passive sampling of SVOCs in water, but has yet to be characterized for passive sampling these chemicals in air. The advantage of using PDMS in its applicability to a wider range of compounds than polyurethane foam (PUF), it is also easier to handle than styrene dibenzyl copolymer (XAD), and it is unobtrusive for indoor deployment. This research presents chemical uptake kinetics of PDMS and PUF for passive sampling of SVOCs in indoor air in the context of occupational, environmental health and safety (OEHs). Calibration studies were conducted to derive passive air sampling rates and to estimate equilibrium times and sampler–air partition coefficients for chemicals. An 8-week long indoor air calibration study for PDMS and PUF disk samplers was conducted from August to September 2013. The experiment was repeated during the same period in 2014 using a larger PDMS. Following strict quality assurance and quality control protocols, all samples from the both studies were extracted and analyzed for polybrominated diphenyl ethers (PBDEs), novel flame retardants (NFRs), phthalates, polycyclic aromatic hydrocarbons (PAHs), and polyorganophosphate esters (OPEs), by gas chromatography–mass spectrometry (GC-MS). Results suggest that the designs of PDMS and PUF samplers are suitable for deployment indoors for a month for the range of chemicals investigated, with the exception of ATE. PDMS sampling rates for NFRs varied from 1.09 m³/day/dm² (PBT) to 2.01 (ATE) while that of partially sheltered PUF was 1.23 m³/day/dm² (PBZB and PBT). For PBDEs, sampling rates of 0.65 (BDE-71) to 0.01 m³/day/dm² (BDE-66) were calculated for PDMS while those for partially sheltered PUF ranged from 0.88 (BDE-17) to 0.99 m³/day/dm² (BDE-99). Sampling rates of PDMS and partially sheltered PUF PAS are in reasonable agreement; however, they were typically 2 to 3 times higher than those of fully sheltered PUFs measured in this study and the literature.

274 Passive sampling as a screening tool in Ireland for new and emerging chemicals

I. Jones, Dublin City University / Chemical Sciences; J. Ronan, Dublin City University; B. McHugh, E. Mc Govern, Marine Institute; F. Regan, Dublin City University / Chemical Sciences

The impact of this study may lie in the establishment of a capability to utilise passive sampling in thromscreening programme in Ireland for WFD. This project pilots the use of passive sampling technology combined with biota monitoring to assess the presence of priority substances in Irish surface waters. The project focuses in particular on new pollutants earmarked as candidates for the Annex X priority substances list under the EU Water Framework Directive (2000/60/EC) e.g. E2 and EE2, pharmaceuticals, pesticides, PFOS etc. This considers the implications for compliance with current and proposed EQS and investigates the potential for incorporating passive sampling and biota testing in future compliance, investigative and trend monitoring. Results of water, biota and passive sampling will be presented together for samples collected in the Dublin catchment. A separate study
on the occurrence of the pyrethroid pesticide cypermethrin was also conducted. Several sites along the River Liffey, Dublin, were sampled for pharmaceutical as well as other organic pollutants. A POCIS device was deployed at each location and water samples were collected at T-0 and T-end. There are a number of potential point sources of pollution to this catchment with 3 wastewater treatment plants in the area. Pyrethroids have a low toxicity relative to other pesticides (specifically the organochlorines) so they have recently been used in place of more toxic pesticides. CONCENTRATION OF ANALYTIC SUBSTANCES IN WATER SAMPLING DEVICES

This study involved collection of water samples alongside PDMS and SPMD samplers. Cypermethrin was detected in high levels in the water and PDMS samplers. Passive sampling devices can be a useful supporting technique in a ‘toolbox’ for monitoring within the WFD and other environmental programs. From the investigation of work to date it is clear that passive sampling can play an important role in screening of waters for emerging contaminants, especially for hydrophobic substances where they could be incorporated into a risk based approach to monitoring. Also, passive sampling has demonstrated that it has a role to play in trend monitoring to illustrate where waters are improving in quality over time, thereby offering the WFD monitoring programme a valuable tool.

275 Passive air sampling of semi-volatile hydrocarbons using polyethylene strips: Mass transfer analysis and calibration with performance reference compounds

P. Grathwohl, Uni Tübingen / Center of Applied Geosciences; A. Akinudire, University of Tübingen; B. Beckingham, College of Charleston / Environmental Geosciences

Polyethylene (PE) is a promising material for passive air sampling of many organic pollutants, yet sufficient understanding of mass transfer kinetics is required. In this study, 50 µm-thick PE strips spiked with performance reference compounds (PRCs) were deployed indoors (without wind protection) and outdoors (in winter and summer with wind protection). The low volatility of the deuterated fluorene (d-FLU) PRC was fitted with the double film diffusion model to calibrate the air-side boundary layer thickness (1.4 mm for unshielded and 3.3 mm for shielded deployments). Losses of higher molecular weight PRCs (deuterated pyrene and benzo-a,anthracene) were very low but they could reasonably be predicted by the calibrated model. Uptake of poly cyclic aromatic hydrocarbons from the atmosphere could be predicted very well while the model yields their concentration in gas phase. Low molecular weight PAHs (e.g. naphthalene) equilibrated in a few hours, whereas the equilibration of the high molecular weight PAHs (e.g. pyrene), was mostly less than 0.1% at the end of deployments (2 weeks) with robust uptake data that integrated concentration and environmental parameters fluctuations. Using the appropriate uptake model allows us to use just one PRC which is particularly beneficial in terms of cost and ease of measurement when uptake to PE is air-side controlled. Models applied here to PAHs can be also transferred to other semi-volatile (emerging) compounds and they allow to calculate the time interval over which the sampler equilibrates, which is a function of their polymer/air partition coefficients (which is strongly correlated to their vapour pressures).

276 Silicone-based passive samplers for monitoring hydrophobic emerging contaminants in the marine environment: calibration and field application

M. Pintado-Herrera, Universidad de Cádiz (Spain) / Department of PhysicalChemistry, University of Valencia / Norwegian Institute for Water Research NIVA; A. Gonzalez-Mazo, University of Cadiz / Physical Chemistry; P. Lara-Martín, University of Cadiz (Spain)

There is a growing interest on knowing the concentration and distribution of a large group of new non-regulated organic compounds (emerging contaminants) in the environment, many of them used in consumer goods, such as antimicrobials, fragrances, UV filters or flame retardants. Some of these chemicals present vapour pressures. Therefore, the use of passive samplers presents a very powerful alternative to estimate aqueous concentrations.

Moreover, these samplers can provide time-integrated concentrations of the analytes in the environment. In this work, the use of single phase sampler, such as low density polyethylene (LDPE) and silicone rubbers (SR) has been tested. The main objective of this work was to calibrate passive samplers to relate the concentrations of several emerging contaminants in the sampler with their free dissolved concentrations in water for some emerging compounds. In this regard, laboratory experiments were performed to establish diffusion, since the transport of analytes from the water to the passive sampler is diffusion-controlled and sampler-water partition coefficients, both are needed to estimate the dissolved concentration of any analyte. Later, SR samplers were chosen and deployed during one year at several sampling stations located within the Bay of Cadiz (SW Spain). In accordance with the results obtained of the laboratory experiments, in general the diffusion coefficient decreased when molecular weight increased. After determining Kpw values, we observed that most of the analytes were positively correlated either with the molecular weight (R² > 0.85). In conclusion, molecular weight is considered a better estimator of log Kpw. Finally, a sampling campaign was carried out in the Bay of Cadiz (SW of Spain). Passive sampling data from this monitoring was compared to those obtained from spot samples collected at the same locations with amber glass bottles.lnLevels of some fragrances (i.e. OTNE) and UV filters (i.e. octocrylene) were predominant over other emerging and regulated contaminants. Seasonal variations were observed for some UV filters (i.e. EHM C and homosolate).

277 Determination of PDMS-water partition coefficients of super-hydrophobic PBTs using empirical maximum solubility

V. J. Schacht, National Research Centre for Environmental Toxicology Entz; S. Grant, The University of Queensland / National Research Centre for Environmental Toxicology Entz; B. I. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; D. Hawker, Griffith University / School of Environment; C. Gaus, National Res. Centre for Environmental Toxicology / Public Health Department

Freely dissolved concentrations (CMw) of contaminants in environmental samples, which are relevant for bioavailability and subsequent exposure, are determined by mass transfer analysis and calibration with performance reference compounds (PRCs) and their air-water exchange. In the water, dissolved concentrations of the ICES 7 point sources of pollution to this catchment.

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Data-driven prioritizing of relevant chemicals for various water types using suspect screening LC-HRMS

R.M. Sierps, A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health

We developed a suspect screening based system in order to prioritize large numbers of compounds for full chemical analysis of relevant water (drinking) water. We based the suspect list on a large number of chemicals authorized on the European market, via various regulations and including hardly studied compounds. This list was applied to a large number of water samples, including effluents, surface waters, groundwaters and drinking waters. We included a TTC based threshold to prioritize the most relevant chemicals that were encountered. The prioritized compounds were compared to the compounds occurring on existing priority lists and to compounds regulated in water quality legislation. Included in the list are chemicals registered under the REACH legislation as well as substances of very high concern (SVHC), chemicals authorized under the Plant Protection Products Regulation and Biocidal Product Regulation and veterinary pharmaceuticals. Mixtures, anorganic, metaloids and non-ionizable compounds were excluded from the list. Only compounds containing N, S, O, or P are considered. The 151 water samples (2007-2014) were well distributed over the Netherlands, and comprised 20 drinking waters, 39 groundwaters, 73 surface waters and 19 effluents. The method provided a fingerprint of prioritized chemicals per water sample and water type. The suspect list covered 5219 individual chemicals. In total 158 masses and 243 suspects in the combined data for algae, crustaceans and fish for subsequent identification of toxicity drivers towards one or more aquatic species. All chemicals prioritized suspects are registered chemicals within REACH. Of the 44 compounds that occur in water quality regulations, only one suspect was prioritized. Of the substances on NORMAN or IAWR/RWIA attention lists and were included in the suspect list, respectively 7.5% and 23.5% were prioritized suspects. This emphasizes the complementarity of the suspect screening approach, in spite of the uncertainties, as compared to the more commonly used target analysis approach. A decrease in summed concentration in terms of IS-equivalents was observed from effluent, to surface water, groundwater and drinking water. A significant decrease can be observed in retention time from effluent towards drinking water, confirming that more polar compounds occur in drinking water.

Bioassay-assisted identification and prioritization of toxicity drivers in the Danube River

K. Tollefsen, NIVA / Ecotoxicological Risk Assessment; T. Gomes, Norwegian University of Life Sciences Research (NIVA); K. Petersen, Norwegian Institute for Water Research; A.R. Almeida, University of Aveiro / Biology; T. Hagåsen, Norwegian School of Veterinary Science (NSV); M. Krauss, T. Schulze, Helmholtz centre for environmental research - UFZ; P. Williams, University of Birmingham / School of Biosciences; P. Antczak, University of Liverpool; Z. Räthova, Masaryk University / Faculty of Science; T. Gomes, RECETOX, J. Slobodnik, Environmental Institute; J. Chipman, The University of Birmingham / School of Biosciences; P. Thomas, CEHTA SAS / Ecotoxicology and Risk Assessment; R. Altenburger, UFC Centre for Environmental Research / Department of Bioanalytical Ecotoxicology

Surface waters contain complex mixtures of inorganic and organic pollutants that enters the environment. As the composition of these complex mixtures are highly variable, assessment of their hazard and risk are often poorly characterized. Efforts to develop a standardized approach to identify individual and cumulative impact of pollutants on organisms are highly warranted. The present work aimed to develop a conceptual approach to 1) identify toxicity drivers to aquatic organisms, 2) provide in-depth knowledge about the Mode of Action (MOA) of key pollutants, 3) identify compounds that may cause combined toxicity in mixtures, 4) formulate a strategy for optimal use of bioassays to characterize the hazard associated with key pollutants and 5) provide linkage to mechanistically-based hazard/risk assessment approaches. This approach will link the existing exposure information from the Danube River (Joint Danube Survey 3, JDS3) to experimental effect data for algae, crustaceans and fish for subsequent identification of toxicity drivers towards one or more aquatic species. All chemicals where subsequently categorized according to chemical group and MOA information to identify suitable bioassays that can be used for biomonitoring purposes. Suitable volumes of bioassays were thereafter used to screen the toxicity potential in large volum solid Phase extraction (LVSPe) extracts and results compared to predicted hazard assessment of these identified MOAs. Efforts to develop new approaches for how to link the available data to concepts such as Adverse Outcome Pathways to support linking chemical and bioassay data to more mechanistically-based hazard (risk) assessment frameworks and aid lab to field extrapolations. Special emphasis were given the identification of ecologically-relevant reference compounds for use in experimental efforts with high-throughput (e.g. in vitro assays) and multi-trophic bioassays (e. g. algae, crustaceans and fish). This approach represent the initial step in a design optimal experimental approaches to decipher complex chemical interactions associated with combined toxicity, and improve bioassay screening approaches in environmental monitoring of European surface waters.

Which chemicals show effects in cell-based in-vitro bioassays? - Are we monitoring the right chemicals?

B.I. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; J.Y. Tang, The University of Queensland / ENTOX; F. Busetti, J. Charrois, Curtin University / Curtin Water Quality Research Centre

Biostochastic tools have been applied worldwide to water quality monitoring for several decades. Most research has focused on surface waters and domestic and industrial wastewaters. In recent years, screening of wastewater and advanced water treatment processes, disinfecting drinking water and recreational waters have emerged. Cell-based assays can then assess even small cytotoxicity or target a specific mode of toxic action or step of the toxicity pathway. Comprehensive risk assessment thus requires a battery of bioassays to cover a range of modes of action and/or toxicity pathways relevant for the water sample to be tested. The goal of this study was to understand which fraction of effect observed in cell-based bioassays can be explained by known chemicals. The answer to this question will be important when deciding whether bioassays are required for water quality monitoring or if the regulated and regularly monitored chemicals are sufficient to assure water quality. For the “iceberg experiments” we mixed the detected chemicals and tested the designed mixtures in bioassays, then compared the results with the biological responses from wastewater treatment plant effluent, treated and recycled water. The results of this study have shown that there is no clear answer to the question of how much of the biological effect can be explained by known chemicals but that it depends on the type of effect. For receptor-mediated modes of action, directivity of the responsive chemicals have been identified, in this study for herbicides and in a previous work for estrogenic chemicals. For more integrative endpoints, such as the oxidative stress response and cytotoxicity, there remain many unknowns and bioassays are clearly needed to get a full picture of the effects of micropollutants.

Development of a miniaturized AMES assay for high-throughput effect-directed analysis of water samples using microfractionation

N. Zwart, Institute for Environmental Studies; T. Hamers, VU University Amsterdam, Institute for Environmental Studies (IVM) / Institute for Environmental Studies; W. Jonker, VU University / BioMolecular Analysis group; C.J. Houtman, The Water Laboratory; J. de Boer, VU University / IVM; J. Kool, VU University / BioMolecular Analysis group; M. Lamoure, VU University, Institute for Environmental Studies / Institute for Environmental Studies

One of the classical toxicological endpoints in analysis of surface water and drinking water is mutagenicity. After its development, the salmonella reversion assay (AMES) has become the most widely used test for genotoxicity. The conventional AMES assay based hazard/risk assessment approaches. This approach will link the processes of drinking water purification depend on the ability to determine the presence of these compounds in surface, ground and effluent water. The application of bioassays like the AMES test together with chemical analysis methods in an effect-directed analysis (EDA) provides a strategy to detect and identify novel mutagenic compounds. However, for rapid toxicity assessment of e.g. water samples, EDA suffers from lack of throughput and is regarded as a laborious, time consuming method. To achieve faster analysis and higher-resolution separation, a high throughput-EDTA (HT-EDTA) method was developed that combines 384-well plate, UPLC based, microfractionation with a miniaturized AMES assay. The applicability of the miniaturized assay will be demonstrated by analysis of selected surface, ground and waste water samples. This application of high-resolution microfractionation together with miniaturized bioassays will open the way to HT-EDTA and will emphasize the advantages of the combined methodology of chemical analysis and bioassays in the analysis of environmental samples, including drinking water.

Sulfonamide Transformation Products: Formation, Analysis, and Residual Antibacterial Activity

M. Majewska, Karlsruhe Institute of Technology (KIT) / Engler Bunte Institute Chair of Water Chemistry and Water Technology; J. Meuswig, Karlsruhe Institute of Technology KIT / Chair of Water Chemistry and Water Technology

Sulfonamide drugs are are widely used in human and veterinary medicine and ubiquitously detected in the aquatic environment. There is increasing concern regarding their potential to alter the composition of environmental bacterial communities and the possible promotion of antibiotic resistance. While the fate and occurrence of the sulfonamides receives increasing attention, the development of transformation products (TPs) only now shift into the focus of environmental risk assessment. Some selected sulfonamide TPs have already been detected in wastewater and surface water alike. However, considering that numerous laboratory studies reported the formation of a variety of TPs, it can be expected that many more sulfonamide TPs occur in the aquatic environment. For the most prominent short-acting human sulfonamide antibiotic sulfamethoxazole (SMX), there are currently more than 20 first daughter TPs known to directly originate from photolysis or biodegradation. SMX certainly is among the best studied sulfonamide antibiotics. Although most likely not all biotic and abiotic transformation reactions reported for SMX are necessarily valid for other sulfonamides, there is much to
suggest that the homologous structure of other sulfonamides exhibit the same reactive sites and thus undergo the same or similar transformations. Therefore, this study presents a data set of more than 20 SMX TPs aiming at identifying the transformation reactions such as acetylation, hydroxylation, nitration or deamination. The elucidation of the product ion spectra during MS/MS experiments allowed for a qualitative determination of suspected TPs for both SMX and of sulfonamide homologues in mineralization and degradation studies. Examples from photolysis and biodegradation are presented. Two new metabolites have been confirmed via synthesized standards. Some of the TPs still contain a pharmacophore-like moiety, and some derivatives incorporate almost the complete SMX parent compound structure. As a consequence, 11 TPs of SMX formed during human metabolism, microbial biodegradation, photolysis, and hydrolysis were tested for their influence on bacterial growth and luminescence inhibition assays. Results show that derivative TPs clearly exhibit antibacterial activity while compound cleavage leads to the loss of this mechanism of action. The acetylated derivatives retain however less than 10% of the effect of SMX on growth and luminescence inhibition.

**Nano**

**Nanoparticle (NP) speciation and its consequences for NP environmental fate and effects (II)**

284 Effects of multigeneration exposure of pristine and sulfidised silver nanoparticles on *C. elegans* and potential recovery

C. Schulz, Centre for Ecology and Hydrology; A. Wamuch, University of Kentucky; O.V. Tsyusko, University of Kentucky / Department of Plant Soil Sciences; J.M. Unrine, University of Kentucky; C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology; D. Spurgeon, Centre for Ecology & Hydrology

As research on the hazard assessment of nanomaterials advances it has become clear that to address their environmental impact toxicity testing needs to go beyond standardised laboratory test and include more environmentally realistic exposure scenarios. To gain a better understanding of their environmental impact experimental designs should aim to include exposure relevant transformed materials as well as longterm exposures to investigate the longterm and transgenerational effects that can occur for some recalcitrant nanomaterials and continual input. In this study potential changes in sensitivity of nematodes to silver ions (AgNO₃), pristine (70 nm, PVP coated) and artificially aged (76 nm, Ag,S) silver nanoparticles on the growth, reproduction and lifespan of the soil dwelling nematode *Caenorhabditis elegans* is studied following exposure over multiple generations (0, 2, 5, and 10) and potential recovery of adverse effects on cessation of exposure. Exposure concentrations for the continuous exposures are based on the reproductive EC₅₀ for AgNO₃ and Ag-PVP; AgNO₃ = 0.15 mg/l, Ag-PVP = 1.5 mg/l, Ag,S = 15 mg/l. Preliminary data analysis showed considerable differences in toxicity between ionic silver and the two silver nanoparticles of AgNO₃ > Ag-PVP > Ag,S. Further initial results showed effects of chronic exposure on the lifespan for AgNO₃ at 2.4 mg/l with lifespans decreasing from 17 ± 1 days in the parent to 8 ± 2 days in the second offsprings generation. Such effects on life-span were not observed for the other treatments. Multi-generation exposure indicated that in the parent generation, there was no significant difference in size between controls and the highest doses (AgNO₃ = 2.4 mg/l, Ag-PVP = 24 mg/l, Ag,S = 120 mg/l) 48 hours after hatching. However, by generation 5, growth and body size showed high sensitivity, such that both were adversely affected by the highest Ag doses. Overall there appears to be an increase in sensitivity over the tested generations, however, this has yet to be confirmed with further data analysis as well as for subsequent generations.

285 Effects of ageing and soil properties on the bioavailability of Zn nanoparticles to *Eisenia andrei*

A. Colman, UGR CIF Q1818002F / edafologia y química agrícola; F.M. Peinado, University of Granada / edafología y química agrícola; C. van Gestel, VU University Amsterdam / Ecological Science

Zinc oxide (ZnO) is one of the most commonly used types of metal-based nanoparticles (NP) which enter the environment with soil being one of the main sinks. In ecotoxicological assays, exposure of earthworms to ZnO NPs and ZnCl₂ resulted in a similar uptake and internal distribution of Zn, suggesting a comparable bioavailability of these NPs. Moreover differences in zinc bioavailability between aged and freshly spiked soils depend on soil properties. Long-term processes (ageing) could reduce ZnO NP bioavailability and toxicity, but it remains unclear if this is due to changes in soil pH or in Zn NP dissolution. It is essential to study the conditions and contribution of NPs to the long-term toxicity of ZnO NPs in terrestrial environments. The aim of this study is to determine the bioavailability of ZnO NPs and the influence of ageing on the Zn toxicity to *E. andrei*, in soils with different physicochemical properties. Three soils with contrasting properties were spiked with ZnO NPs (concentrations close to the EC10 and EC50) and ZnCl₂ (EC50). Soils were moistened and incubated for 196 days under controlled conditions (ageing). At different time points, available Zn levels in the soils were determined by extraction with 0.1 M copper nitrate ([Cu(NO₃)₂]₃H₂O) and pore water analysis. Earthworm toxicity tests (OECD, 2004) were performed with survival, weight change and reproduction as the endpoints. Zn concentrations in the copper nitrate extracts ([ZnCu], pore water [ZnPw] and in earthworm tissues ([ZnE]) were measured by ICP-MS. Results showed that soil pH values decreased upon addition of ZnCl₂, while it was increased by the addition of ZnO NPs. Copper nitrate extracted almost all the zinc (available Zn), while Zn concentration in PW was lower than 17% of the Zn added. The highest available Zn levels were found in soils spiked with ZnCl₂. In general, extractable Zn levels significantly decreased with ageing of the soils, for both ZnCl₂ and ZnO NPs. The lowest available Zn levels were found in the alkali treated soil. Earthworm reproductive toxicity was assessed via ZnCl₂ due to its highly bioavailable and soluble ionic Zn form. Reduction compared to controls showed a different behaviour depending on the test soil for the different ageing periods suggesting that Zn bioavailability (and toxicity) is dependent on ageing and soil properties. Further analyses of the data are needed to relate toxicity and soils properties in order to assess the risk of nanoparticles over time.

286 Speciation matters—Bioavailability of silver and silver sulfide nanoparticles to *alphafila* (Medicago sativa)


Silver nanoparticles (nano-Ag(0)) are incorporated into consumer products which leads to an increased demand to understand the environmental fate of nano-Ag(0) and their important environmentally-transformed analog, silver sulfide nanoparticles (nano-Ag,S). Nano Ag(0) and nano-Ag,S in waste water treatment plants and landfills applied as biosolids present a potential exposure route for terrestrial and aquatic plants. In the present study, the uptake of silver into alfalfa (Medicago sativa) plants was quantified and visualized upon hydroponic exposure to a suspension of nano-Ag(0), nano-Ag,S, and as a control, a silver ions solution (Ag⁺). The total silver uptake was determined in dried roots and shoots, and the spatial distribution of silver was investigated using microscopy and secondary ion-based X-ray techniques. Both nano-Ag(0) and nano-Ag,S were bioavailable in the root systems and exhibited limited translocation of silver into the shoot system. X-Ray fluorescence (XRF) mapping suggests that the mechanism of silver uptake for nano-Ag(0) is similar to the mechanism for dissolved silver ions. Alternatively, the nano-Ag,S was found to adhere to the external surface of the root with limited incorporation into the root tissue, and was associated with the formation of nano-SiO₂ particles (phyloliths) in the cytoplasm of these plants. The overall phytoavailability and capacity of the nano-Ag-S to translocate throughout the plants remains similar to the pristine nano-Ag(0), despite lower silver ion concentrations associated with the less soluble Ag,S particles.

287 Using surface modified CdTeS/ZnS nanocrystals (QDs) as nanotracers in complex waste matrices

F. Part, University of Natural Resources and Life Sciences, Vienna / Department of Water Atmospheric Environment WAU; C. Zaba, University of Natural Resources and Life Sciences Vienna / Department of Nanobiotechnology DNBIT Institute for Synthetic Bioarchitectures; M. Oberwalder, University of Natural Resources and Life Sciences Vienna / Department of Chemistry DCH Division of Analytical Chemistry DCHAC; M. Huber-Humer, University of Natural Resources and Life Sciences Vienna / Department of Water Atmospheric Environment WAU Institute of Waste Management ABFBOKU; E.K. Sinner, University of Natural Resources and Life Sciences Vienna / Department of Nanobiotechnology DNBIT Institute for Synthetic Bioarchitectures

Engineered nanomaterials (ENMs), such as nano-TiO₂, -SiO₂, carbon nanotubes, fullerenes, Quantum Dots (QDs), are already applied in many consumer products. The area of applications ranges from cosmetics, construction materials to and electronic equipment. In the future, the variety and production volumes of ENMs can only be expected to increase. CdS, CdSe or InP QDs are used for their semi-conducting and fluorescence properties in thin solar cells and LED background lightings. Surface modified QDs are utilized as fluorophores and biomarkers in medical applications. In order to improve the performance of QDs and add desirable properties they can be surface modified. E.g., the photoluminescence quantum yield of QDs can be enhanced by reducing surface defects using capping agents or CdS shells. A variety of surface modifications influence the photoluminescence—e.g. by using N-acetyl-L-cysteine (NAC). Carboxyl functionalised groups lead to a higher surface charge and may increase cellular QD uptake. In general, more research is needed to assess the eco-toxicological effects of QDs. Our project shows the potential of QDs to be used as nanotracers for studies about the fate and behaviour of ENMs in complex matrices, such as liquid waste samples. In this study, we synthesised QDs and added them with carboxyl-terminated coatings. QDs were characterised by using dynamic light scattering (DLS), transmission electron microscopy (TEM) and inductively coupled plasma mass spectroscopy (ICP-MS). These nanotracers were spiked to landfill leachates, one potential QD source. The complex leachate matrix, containing QDs, was screened by ICP-MS and fluorescence analysis.

288 The bioavailability and toxicity of nanoparticles in biosolids to earthworms

F. Lahive, Centre for Ecology & Hydrology (NERC); M. Matzke, Centre for Ecology & Hydrology (NERC) / Molecular Ecotoxicology; A. Romero, UGR (CIF)
The criticality issue to be addressed in the current LCA and the need for a broader perspective. The South Africa is typically known for its platinum, gold and coal. Further, based on international competition, it ranks number one and two for chrome/ferro-chrome, platinum group metals (PGMs), vermiculite and antimony, manganese ore, titanium minerals, vanadium, zirconium respectively. South Africa also ranks as having the highest reserves for chrome ore, fluorspar, manganese and PGMs. There is also a move to start larger exploration and mining of rare earth minerals.

291 From Scarcity to Resource Security Indices: an approach to assess the geopolitical security of resource supply

E. Gemechu, University of Bordeaux / Institute of Molecular Sciences ISM; G. Semisch, University of Brest / The Life Cycle Group CyVi

There is an increasing concern among citizens, businesses and governments over the security and sustainable supply of resources mainly due limited availability. The resource challenges are very complex in nature, therefore, assessing the geopolitical resource security and sustainability needs a new perspective and related analytical approaches to cope with it. Life cycle assessment (LCA) has been a standardized approach used to assess the direct impact from the use of resources. For some time now, there has been a discussions within the LCA community on how the resource issue to be addressed in the current LCA and the need for a broader perspective. The direct impact from the use of resources in LCA is mainly focused on the geological availability, which has a long-term time perspective. Other aspects such as supply chain risks due to short-term geopolitical actions as well as social and economic factors are not well covered in the current framework. This study investigates the bioavailability of PGMs, vermiculite and antimony, manganese ore, titanium minerals, vanadium, zirconium respectively. South Africa also ranks as having the highest reserves for chrome ore, fluorspar, manganese and PGMs. There is also a move to start larger exploration and mining of rare earth minerals.

290 Setting targets on the environmental footprint of nations using planetary boundaries

R. Frischknecht, S. Buesser, tweez Ltd.; C. Nathani, Rueter Partner

Domestic environmental impacts may be reduced by outsourcing potentially highly polluting activities, such as heavy industries and agriculture, to another country. To what extent such measures or effects may help to fulfill and comply with national environmental targets and policies, outsourcing and maintaining polluting activities abroad does not lead to environmental improvements on a global scale. That is why the development of consumption-based environmental impacts of Switzerland from 1996 to 2011 was quantified. A simplified physical model was used which quantifies domestic environmental impacts and subtracts environmental im-pacts related to exports combining foreign trade statistics with life cycle assessment data. Environmental impacts are quantified with the several single score methods such as the Swiss eco-factors 2013 based on the ecological scarcity method and with several midpoint indicators. This presentation focuses on eutrophying impacts of Swiss consumption. Marine eutrophication is quantified as an indicator of the amount of nitrogen emitted and reaching marine environments. Consumption-based marine eutrophication impacts increased by 8.5% since 1996 because emissions due to foreign trade increased substantially while domestic emissions showed hardly any decrease. At the same time the share of eutrophying substances emitted abroad increased substantially. Hence, regulating eutrophying substances within the country does not suffice in open economies such as Switzerland. The planetary boundary concept is applied on consumption-based marine eutrophication. The knowledge on consumption-based environmental impacts and its domestic share can be applied on target setting. National reduction targets are applied on the domestic emissions whereas a global planetary boundary is used to define the reduction target for eutrophying substances emitted abroad. It was shown that Swiss consumption-based marine eutrophication of about 118 000 t of nitrogen equivalents should be reduced by nearly 60%. 30% of the reduction needs to be implemented in Switzerland, the remaining 70% of the required reduction should be achieved abroad.
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Quantifying spatially derived carrying capacity occupation: Framework for characterisation modelling and application to terrestrial acidification
A. Bjorn, Technical University of Denmark / Department of Management Engineering; M. Margni, Ecole Polytechnique de Montreal / Mathematical and Industrial engineering; p. noy; C. Bulle, CIRIAG - ESG - UQAM / Strategy corporate social responsibility; M. Hauschild, DTU Management Engineering
The concept of planetary boundaries and the challenge of absolute constraints. The paper is based on an analysis of the current carrying capacity footprint method and may be compared to the availability of land or water. The framework was applied to the terrestrial acidification impact category. The geochemical steady-state model PROFILE was used to quantify carrying capacities as deposition levels corresponding to an acceptable change of natural pH at a 2.0x2.5° resolution at the global scale. Carrying capacities were then combined with atmospheric fate factors of acids by staying under the carrying capacity to derive PCFs that were applied to an average emission inventory for the annual electricity consumption of a household in 100 random global locations. To evaluate the consequence of using the CFs in a comparative assessment the 100 impact scores were ranked and compared to the corresponding ranking when using existing CFs based on marginal impacts above carrying capacity on the same inventory. The difference in ranking reflects the different natures of the two sets of CFs: The existing CFs are aligned with consequential thinking and concerned with marginal changes above carrying capacity, while our derived CFs are aligned with attributional thinking and concerned with the occupation of carrying capacity. This work shows the viability of spatially derived absolute sustainability assessment, i.e. assessments where impacts are compared to sustainable levels of impacts. This can become an important supplemental to the predominant relative environmental assessments, where impacts of different product systems are compared.

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Ethical perspectives on planetary boundaries and LCIA
B. Woudstra, Aalborg University / Danish Centre for Environmental Assessment; M. Brandão, 94 Pahiatiu Street
The concept of planetary boundaries has resurfaced the discussion on carrying capacity and target setting for overall environmental impacts. This concept is now widely used in both academic and political decision-making circles, particularly in climate policy after the Copenhagen Accord (2009) made explicit the political aim of respecting different natures of carrying capacities and occupation of carrying capacity. This work explores the usefulness of this concept for decisions that carry trade-offs. Some authors see the concept of planetary boundaries as complementary to and compatible with the concept of sustainability. We argue here that the concept of planetary boundaries has a different ethical basis than the concept of sustainability, and that this is parallel to the incompatibility between Distance-To-Target and Damage approaches in life cycle impact assessment (LCIA). The concept of sustainable development implies trade-offs between different needs aiming at optimising production and consumption systems in order to maximise utility within existing constraints, and therefore is firmly grounded in utilitarian ethics. The concept of planetary boundaries, on the other hand, implies the setting of targets not to be exceeded, and thus shifts the focus from relative trade-offs to absolute constraints, and can therefore be seen as an expression of a rule-based ethic. We argue that the choice of weighting methods in LCIA, and the choice between sustainable development and planetary boundaries, is fundamentally an ethical and political choice, and should not be disguised as science. The main relevance of the notion of planetary boundaries is that it raises the importance of ethical and political questions, and the need for a tipping point in the utilitarian damage approach, without the need to introduce targets, by adding this concern as a separate impact category with its own metric of carrying capacity occupation. This work shows the viability of spatially derived absolute sustainability assessment, i.e. assessments where impacts are compared to sustainable levels of impacts. This can become an important supplemental to the predominant relative environmental assessments, where impacts of different product systems are compared.

Midpoint or single score for decision making? (I)

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The use of uncertain product footprints in a comparative context
P.J. Henriksson, Leiden University / Institute of Environmental Sciences; L. Guinee, University of Leiden / Institute of Environmental Sciences; R. Heijungs, Leiden University / Faculty of Economics and Business Administration Department of Econometrics and Operations Research
In response to growing awareness of climate change, requests to establish product carbon footprints (PCFs) have been increasing. PCFs are life cycle assessments (LCA) restricted to just one impact category, global warming. PCF studies generate life cycle inventory (LCI) results, listing the environmental emissions of greenhouse gases (GHGs) from a product's lifecycle, and characterize these by their global warming potentials, producing PCFs that are commonly communicated as point estimates. In the presence of uncertainty, these point estimates are not sufficient for decision-making, and multiple publications have suggested methods for communicating these point values more effectively.

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How to use the available knowledge in LCIA to help understand relative importance and relevance in trade-offs and help making an informed decision: Application to the case of PEF/OEF pilots
S. Humbert, Quantis
The life cycle impact assessment method recommended by the European Commission for the Product Environmental Footprint (PEF) and the Organisation Environmental Footprint (OEF), based on ILCD, is a list of 15 indicators at midpoint level in LCA. These references are operational and their meaning can easily be communicated to practitioners and decision makers. Yet they do not capture potentially important spatial variations in carrying capacities. To overcome this weakness we propose to integrate carrying capacity in characterisation factors (CFs) as an alternative to using carrying capacity as reference information in normalisation references. We developed a generic mathematical expression for a spatially differentiated CF, which allows expressing impact scores as occupation of carrying capacity in units of km²/year. This metric resembles that of the ecological footprint methodology and may be compared to the availability of land or water. The framework was applied to the terrestrial acidification impact category. The geochemical steady-state model PROFILE was used to quantify carrying capacities as deposition levels corresponding to an acceptable change of natural pH at a 2.0x2.5° resolution at the global scale. Carrying capacities were then combined with atmospheric fate factors of acids by staying under the carrying capacity to derive PCFs that were applied to an average emission inventory for the annual electricity consumption of a household in 100 random global locations. To evaluate the consequence of using the CFs in a comparative assessment the 100 impact scores were ranked and compared to the corresponding ranking when using existing CFs based on marginal impacts above carrying capacity on the same inventory. The difference in ranking reflects the different natures of the two sets of CFs: The existing CFs are aligned with consequential thinking and concerned with marginal changes above carrying capacity, while our derived CFs are aligned with attributional thinking and concerned with the occupation of carrying capacity. This work shows the viability of spatially derived absolute sustainability assessment, i.e. assessments where impacts are compared to sustainable levels of impacts. This can become an important supplemental to the predominant relative environmental assessments, where impacts of different product systems are compared.

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A distance-to-target weighting set to assess the progress towards EU 2050 targets
S. Sala, European Commission DG Joint Research Centre / Institute for Environment and Sustainability; V. Castellani, European Commission DG Joint Research Centre / Institute for Environment and Sustainability; L. Benini, JRC Institute for Environment and Sustainability - European Commission / Sustainability Assessment unit
Normalisation and weighting are optional steps according to ISO14040. However,
in the context of LCA in support to policy they can help: i) to identify the most relevant impact categories in a given region or for a given product, i.e. to define Product Category Rules (PCR) for product labelling (as in the case of Product Environmental Footprint – PEF – of products (EC, 2013)); ii) to address eco-innovation policies and strategies towards the most effective solutions for decoupling. Distance-to-target (DTT) methods are weighting methods aimed at assessing the degree of an existing situation from a desired state (the target). Weighting factors in distance-to-targets methods could be based on principles which are performed on normalisation factors (NFs)’ developed for Life Cycle Assessment (LCA). The NF’s express the total impact of a reference region for a certain impact category (e.g. climate change, eutrophication, etc.) in a reference year, whereas weighting reflects a process of giving different relative importance to impacts based upon criteria. The weighting factor is defined for each environmental impact category as the ratio between the actual impact and the target impact. The targets impacts can be the desirable level of impact foreseen by policy targets or physical targets or thresholds not to be trespassed, as in the case of planetary boundaries. In the present study, the target value for each impact category is derived from EU policy targets for 2020. Therefore, normalization reference values are the NF’s for the current scenario (referring to year 2010) and targets values are derived from the application of policy targets for 2020. The methodology implemented for calculating NFs2020 and related weighting factors (WFs) applies a distance to target method based on binding and non-binding EU policy targets for 2020. The methodological steps are: 1. Identification of binding and non-binding EU targets for 2020; 2. Development of the inventory in which targets are herein reduced; 3. Calculating overviews of weighting fac tors are modeled on binding and non-binding targets, respectively. The methodology allowed to derive DTT weighting factors at the EU scale based on policy targets. The resulting sets present strength and limitation that will be discuss especially in light of theri use for policy support.

298 Single score methods and midpoint indicators: a dream team for an effective environmental policy
S. Buesser, R. Frischknecht, treexe Ltd.; C. Nathani, Ruetter Partner

Human societies consume more and more non-renewable resources, and several renewable resources are being depleted beyond their capacity of regeneration. That is the reason why measures are required to lower the environmental impacts of human society. In order to do so national authorities need to know the actual situation regarding emissions of pollutants and extraction of resources. Comprehensive national environmental statistics are one important prerequisite. Besides, it is also important to know the levels and trends of the environmental impacts related to the traded commodities and services. The environmental impacts of products and services consumed in a country need to be quantified along their entire life cycle disregarding national borders. Finally, national action plans targeted to improve the environmental impacts of a nation’s consumption require prioritisation. Single score life cycle impact assessment methods in combination with midpoint indicators are one mean to support this process and help identifying targets that are herein reduced. Calculating overviews of weighting factors are modelled on binding and non-binding targets, respectively. The methodology allowed to derive DTT weighting factors at the EU scale based on policy targets. The resulting sets present strength and limitation that will be discussed especially in light of their use for policy support.

299 Analysis and comparison of monetarization methods with application to two paint formulations
J. Lindberg, JVL Swedish Environmental Research Institute; K.A. Haldldn, Akzo Nobel / Sustainability; M. Lindblad, H. Tekie, T.V. Rydberg, JVL Swedish Environmental Research Institute

Translating environmental impact indicators into economic cost burden for present and future societies, i.e. monetarization, is one available approach for achieving single score results in Life Cycle Assessment (LCA). A study was conducted, with the aim to explore different monetarization methods and apply them to two decorative paints, a former paint formulation and a new paint formulation, in order to investigate the impacts on single scores level. To present the result on single score level has its pros and cons in relation to midpoint level, where the single score gives clear result for example when comparing products, but on the other hand is based on subjective value judgment. The three monetary weighting methods used in the study were Environmental priority strategies - EPS, Stepwise and Ecovalue.

There are many aspects of a weighting method and different methods could be suitable in different application areas and decision situations. The three methods were selected from different criteria for example environmental representativeness, valuation attached to midpoint and endpoint level, principles of the weighting methods (what are the values based on, whose values are used and how the values are derived). All methods covered a broad range of environmental impacts or damages, some midpoint and endpoint impacts are represented and the methods are based on different principles. The aim of this is to illustrate the result from different angels and to overcome data gaps. The overall result showed differences with a factor of three between the method with lowest (Ecovalue) and highest (EPS) environmental costs for the specific paints in the study. The largest contributions to the environmental costs were given by the climate change category or related damages, but also particulate matter formation or related impacts gave a high contribution for the three methods. In the course of the study, it was observed that the methods are typically based on marginal cost estimates. The implications of this choice in relation to attributional or consequential modelling in LCA are discussed, as it can be argued that an average cost model would be more consistent with the conventional attributional LCA modelling practice.

300 The Method of Ecological Scarcity: Adaptation to German Framwork Conditions as Tool for Environmental Management
I. Schebek, Technische Universitaet Darmstadt / Material Flow Management and Resource Economy; S. Abhe, Syrcoc; N. Jansky, TU Darmstadt / Institute IWAR

In the current debate for the development of environmental policies, a fact is to be noted that the current data on respective substance or material flows. This approach is in line with the need for ecological scarcity to be used as a tool for the implementation of political targets for specific target setting of stakeholder groups. In this sense, eco-factors are clearly most suitable specifically for use in environmental management. Here, also the continuous agenda setting within the Audit Schedule may be supported by regular up-dating of eco-factors.

Assessment of risks posed by systemic insecticides to hymenopteran pollinators: from lab via (semi-)field to landscape scale (II)
U. Heinbacher, JKI / A; M. Stahler, JKI / Institute for Ecological Chemistry Plant Analysis and Stored Product Protection; D. Schenke, Institute for Ecological Chemistry Plant Analysis and Stored Product Protection; J. Pistorius, Julius Kuhn-Institut

Seeds of many crops are treated with insecticides to protect young seedlings against pests. Dust drift during sowing of pesticide treated seeds - emission in adjacent areas and effects on honey bees
U. Heinbacher, JKI / A; M. Stahler, JKI / Institute for Ecological Chemistry Plant Analysis and Stored Product Protection; D. Schenke, Institute for Ecological Chemistry Plant Analysis and Stored Product Protection; J. Pistorius, Julius Kuhn-Institut
Petri dishes on bare soil only fell slowly with increasing distance (fig 1). Generally higher Heubach values or higher amounts of a.s. in dust resulted in higher drift values. Residues of a.s. (in g/ha) in adjacent crops were up to about 5 times higher compared to values of Petri dishes (in g/ha) in open areas at 1 m distance in several experiments. This points out a filtering capacity of adjacent plants. Residues in flowering parts of the adjacent crops were high and reached critical levels in some experiments. In these cases the exposure of honey bees was so severe that the bee exposure experiments were conducted parallel to drift experiments and showed clear bee effects in some of the experiments. Bee exposure experiments were conducted parallel to drift experiments and showed clear bee effects in some of the experiments. E.g. in the autumn 2013 experiment for few days some increased bee mortality was observed in tents with honeybees placed directly adjacent to the drilled area after sowing. No such effect was visible during open field exposure of honey bees in this experiment. No effects on bees were observed in the 2014 experiment, neither in tent nor in open field exposure. Risk mitigation for pesticide treated seeds seems possible with an improvement of the seed coating quality regarding content of loose dust, maximum amount of active substances in dust and with improvements of sowing techniques.

302 Imidacloprid and immunity: abumble bee perspective
E.J. Collison, Food and Environment Research Agency / Biosciences College of Life and Environmental Sciences; H. Hird, The Food and Environment Research Agency; C.R. Tyler, Biosciences College of Life and Environmental Sciences; J. Cresswell, Exeter University

Modelling honey bee immunity is likely contributing to declines in bee health, including pesticide application and the spread of pathogens and parasites. Recent recommendations for the risk assessment of plant protection products on bees have included the need for a better understanding of pesticide-disease interactions and the development of biomarkers to evaluate sublethal effects of pesticide exposure, such as immune system changes. Studies have begun to investigate the biological mechanisms underlying pesticide-disease interactions in honey bees, and there is some evidence that exposure to neonicotinoid pesticides impairs the honey bee immune response. However, there has been no investigation of neonicotinoid effects on the bumble bee immune response despite recognition that the sensitivity to neonicotinoids, and endpoints appropriate for risk assessment, could differ between bee species. Here a laboratory study was set up to investigate the effects of a chronic oral imidacloprid exposure on the exposure on the immune response of the bumble bee (Bombus terrestris lataud). We tested whether imidacloprid exposure impaired the ability of workers to respond to an artificial immune challenge with E.coli lipopolysaccharides (LPS), measuring two components of the insect immune response: phenoloxidase enzyme activity (associated with a melanisation response), and antimicrobial protein (AMP) activity. Neither immune challenge nor imidacloprid exposure was found to affect phenoloxidase enzyme activity in the bumble bee immune response. Injection with LPS lead to a significant induction of antimicrobial protein activity compared to unchallenged (naïve) controls, likely due to a combination of wounding during injection and response to the bacterial LPS molecules. The induction of AMP activity in response to LPS injection was found to be reduced in a way proportional to the highest concentration of imidaclopid (125 µg/L², 110 ppb), but there were no significant effects on AMP activity at more field relevant imidacloprid exposures. We conclude that the phenoloxidase response to immune challenge remains difficult to interpret and may be of little value to pesticide risk assessment. Our data also question the value of AMP activity to understand the effects of toxicants in the bee hive it is important to fully understand their fate within bee colonies. Spatial distribution of plant protection products is calculated between compartments in the honey bee colony – a needful extension to an approach that investigates the distribution of pesticides in honey bees and their movements in order to describe the substance distribution in the honey bee colony in its full extent. The partition will be described considering the specific “architecture” of the honey bee comb cells, the chemical constituents of the different matrices and the partition coefficient of the substances of which the distribution behaviour is examined. The methods of investigating the partition behaviour of substances between the different matrices in the honey bee colony are compared to literature data. The calculated partition behaviour is in agreement with data from literature. The model generates results that correspond with values obtained from examined hives. As a submodel of a general model that describes the toxicokinetics of substances in the honey bee colony, it will perform the task of calculating the substance transfer between storages as well as brood and wax.

305 Pollinator profile of the novel Cytanranilprole insecticide
A. Dinter, DuPont de Nemours (Deutschland) GmbH / Crop Protection; A. Samel, DuPont Crop Protection / Ecotoxicology

Cytanranilprole (DPX-CIFAR™ Cyazypyr®) is an anthranilide diamide insecticide (IRAC Group 28) and the first to control a cross-spectrum of chewing and sucking pests. Cytanranilprole is a systemic insecticide and products containing cyantranilprole are optimized for foliar or soil applications and are effective on a wide range of crops (i.e. vegetables and top fruits). Application rates vary for different pests and crops between 12.5 to 150 g cyantranilprole/ha with up to 24 applications per crop. The distribution of cyantranilprole is governed by worker behavior and their movements in order to describe the effects of cyantranilprole in the honey bee colony – a needful extension to an approach that investigates the distribution of pesticides in honey bees and their movements in order to describe the substance distribution in the honey bee colony in its full extent. The partition will be described considering the specific “architecture” of the honey bee comb cells, the chemical constituents of the different matrices and the partition coefficient of the substances of which the distribution behaviour is examined. The methods of investigating the partition behaviour of substances between the different matrices in the honey bee colony are compared to literature data. The calculated partition behaviour is in agreement with data from literature. The model generates results that correspond with values obtained from examined hives. As a submodel of a general model that describes the toxicokinetics of substances in the honey bee colony, it will perform the task of calculating the substance transfer between storages as well as brood and wax.
toxicity that appear to be linked to exposure of bees to dust from neonicotinoid seed treatments, but these are unrelated to the kind of chronic toxicity and effects from sublethal exposure that have been postulated. The author performed a causal analysis of reported declines in bee colonies with a special emphasis on the role of neonicotinoid insecticides.

Improving approaches for difficult non-standard risk assessment of chemicals


Naturally-occurring substances can be monocoenist (e.g. metal ions or organic molecules) or multi-coenist/UCVB (e.g. plant extracts). It can be misleading to assume that all naturally-occurring substances are benign, or that organisms are unaffected by them. An approach for method development (REACH) has been developed mainly for anthropogenic substances, and could yield misleading results when applied to naturally-occurring substances without serious consideration of the assumptions. Risk assessments of organic naturally-occurring substances present their own scientific challenges when compared to anthropogenic substances. The substances are inevitably already present in the environment and the potential risks in increasing exposure to these substances over background levels need to be considered. This paper covers substances that: occur naturally in the environment, are essential nutrients occur endogenously i.e. are present in organisms including humans from metabolic processes. It addresses the following: - The relationship between the natural origin of the substance and risk assessment strategy. - How an assessment is performed when the amount released into the environment is below the background level already present. - How to deal with PNECs at concentrations which are below the background level in the environment. - For man via the environment, how to deal with the issue of mammalian DNEL at concentrations which are both above the safe level for substances that are essential nutrients or which are natural metabolites in the body. The following steps have been identified, and will be exemplified: 1. Identify and characterise the natural and metal compounds the evaluation of both short term and long term. 2. If we cannot determine the background concentration of the substance occurring naturally in the environment and in humans 4: Identify the uses of the substance that will likely lead to an increase in exposure above background concentration levels in the environment and in humans 5: Assess exposure (including background levels): - Obtain hazard property data 7: Interpret the hazard data relative to the background concentration levels 8: Review and finalise the assessment strategy 9: Characterise risk. In respect of properties, biodegradability presents particular problems because standard studies are not always appropriate and other information from known metabolic pathways should be included.

308 An Integrated Fact Sheet for the classification of complex inorganic materials for environmental hazard D. Heijerick, ARCHE; V. VEROUGSTRAETE, Eurometaux / EHS; B. Davies, ICMM; M. Hvaertsoo, Eurometaux; M. van Gheluwe, ARCHE

The general strategy for classifying chemical substances, compounds and mixtures for the environment is below the background level already present. - How to deal with PNECs at concentrations which are below the background level in the environment. - For man via the environment, how to deal with the issue of mammalian DNEL at concentrations which are both above the safe level for substances that are essential nutrients or which are natural metabolites in the body. The following steps have been identified, and will be exemplified: 1. Identify and characterise the natural and metal compounds the evaluation of both short term and long term. 2. If we cannot determine the background concentration of the substance occurring naturally in the environment and in humans 4: Identify the uses of the substance that will likely lead to an increase in exposure above background concentration levels in the environment and in humans 5: Assess exposure (including background levels): - Obtain hazard property data 7: Interpret the hazard data relative to the background concentration levels 8: Review and finalise the assessment strategy 9: Characterise risk. In respect of properties, biodegradability presents particular problems because standard studies are not always appropriate and other information from known metabolic pathways should be included.

310 Non-standard risk assessment - exploring the boundaries A. Kassie, Aperion team NV; C. Vandenbergrouck, Aperion-Team; H. Moerman, F. Van Anbroeck, Aperion-team NV

The fundamental principles of risk assessment of chemicals is well established, and in the framework of REACH, detailed guidance is made available by the European Chemicals Agency (ECHA) on how to conduct a ‘standard’ chemical safety assessment. Nevertheless, for the chemical safety assessment of more complex cases, the standard principles often are not directly or not fully applicable, and expert judgement is required to come to a conclusion regarding the thresholds of safe use (DNELs, PNECs), the PBT status, or for the determination of relevant exposure levels. Specific physicochemical properties of the substance, such as a high volatility, a low water solubility, a high tendency to adsorp to solid matter, et cetera can easily lead to non-standard risk assessments as these properties require non-standard test designs and possibly even non-standard ways interpreting the data, which is not always foreseen in the available guidance/legislation. The risk assessment is even prone to an additional level of complexity when it regards complex substances containing a high number of (similar or unsimilar) chemical constituents. In those cases, the assessment can be hampered by the difference in physicochemical, toxicological and/or ecotoxicological properties of each constituent. Consequently, different constituents could exert different toxicokinetic behaviour (e.g. adsorption, distribution, accumulation) and lead to different metabolites. Likewise, the environmental fate of the constituents may be different, e.g leading to differences in biodegradability or a different distribution between the environmental compartments. In this presentation, we will discuss two (anonymised) cases in which the standard risk assessment principles could not be applied. The first case deals with a substance of complex composition. The substance contains 30+ constituents with a variable but low water solubility. The second case deals with a PBT assessment for an organic substance that has a high tendency for adsorption. The challenges faced were the following: (i) How to design a reliable test?, (ii) Will the adaptations to the standard approaches be accepted in a regulatory context?, (iii) How do we meet the regulatory deadlines while time for development of a non-standard test is not foreseen in the regulation(s), (iv) Proportionality. The described case studies will address the components of the UVCB as though these components were single substances. The strategy has three main strands: 1. Characterising the substance and identifying the HBs and possible constituents. 2. Compile relevant physical-chemical, fate and effect properties for HB constituents 3. Estimate environmental emissions and the environmental exposure to the constituents. Based on these data and the assumption that there is common mode of action, narcosis, the risks of the components are summed thus giving the overall risk of the product, under assessment. Obviously, the CONCAWE approach described above, only addresses the environmental risks for the whole product that arise from normal use (i.e. spillages are excluded). As such the talk will also address how the behaviour of the whole product differs from that of each constituent and thus the case that arise addressing the whole product with respect to the potential for erroneous estimation of the environmental risks. The key questions that come from this approach that will be touched on during this presentation are: - How is uncertainty in the composition of a product addressed both in terms of absolute differences and on the impact of varying HBs? - Where there are many thousands of possible constituents, what uncertainty is introduced by the simplification of HBs to only a few structures? - Can uncertainties in emissions be quantified and if so how do they get addressed?
Registants of substances under the REACH regulation are obliged to carry out a safety assessment for their substance if the manufactured or imported amount exceeds 10 tons per year. If the substance meets the criteria to be classified as hazardous the safety assessment must include exposure assessments for all uses of the substance and a corresponding risk characterisation. The assessment is expected to cover the whole life cycle of the substance. The exposure assessment shall take into account transformation and/or degradations products of the substance that may occur during use or after release into the environment. A substance registered under REACH may consist of various constituents with a narrow or wide range of properties, it may vary in its composition or forms, and the corresponding hazard profile and the use pattern of the substance may be different for different forms or compositions. Consequently a safety assessor under REACH may be confronted with relatively complex assessment cases and transparent documentation in the registration dossier may be a challenge. In order to improve transparency of assessments in the future ECHA has developed the so called “assessment entity” approach aiming to support a transparent organisation of assessment data for substances with a more complex chemistry. The “assessment entity” is a wrapper [container] for a set of substance property data (across endpoints) used for assessment purpose. It enables the identification and tracking of one consistent dataset per exposure assessment when two or more compositions/constituents are to be taken into account in the assessment separately from each other. This approach will be incorporated in the new generation of IUCLID and Chesar, ECHA’s tools for registrants to report data and to assess hazards, exposure and risks. The new functionalities will support the definition of assessment entities and will enable the segregation of consorts between various datasets that form a safety assessment making the compositions/forms of a substance or groups of constituents, the corresponding substance property and hazard profiles, the relevant uses, the conditions of use and the corresponding exposure estimates.

312 The Application of Reactivity Drive Profiling to Identify Chemicals of Higher Concern M.A. Bonnell, Environment Canada / Ecological Assessment Division; O. Mekenyen, Prof Dr Assen Zlatarov University / Laboratory of Mathematical Chemist
The ultimate goal of chemical evaluation is to answer the hypothesis question of whether a chemical has the potential to cause adverse effects in target organisms. This question has been answer both in the near field (local environment) as well as the far field (remotely or globally distributed). The prioritization and assessment of organic chemicals has often been approached using hazard properties (i.e., PBT, vPvB), which because of the history and domain of the criteria for these properties, may not adequately address some reactive and potentially quite potent chemicals that are highly soluble in water (e.g., pharmaceuticals). It can also be argued that the criteria for these properties better capture chemicals that can exist in the far field (e.g., air half-life >2 days, water half-life >182 days) and may not address chemicals in the near field that are “continuously present” and yet potent beyond baseline narcosis. As a consequence there is a very real danger of making false negative errors of omission when using Type II error driven decision-making on the certain chemicals. Here we present an approach that compliments PBT analysis with in silico mechanistic profiling to better address reactivity, ADME and the tautomeric properties of a chemical. The anionic dye/drug addictive/insecticide Phloxine B, will be used to demonstrate this approach particularly because it would not be captured as a PBT or vPvB chemical in the situation of no empirical data. Two case studies will be presented to reveal possible outcomes of risk conclusions: (1) PBT profiling approach assuming no data, and (2) enhanced PBT profiling to include reactivity and ADME profiling also assuming no data. These scenarios are then compared with available empirical data including bioaccumulation and ecotoxicity testing in a soil organism. Recommendations are made as to how enhanced hazard property profiling can be achieved by combining PBT and mechanistic approaches to avoid Type II for these “difficult to assess” substances.

Soil and water pollutants’ assessment, monitoring and remediation (II)

313 Effects-directed approach to assess changes in PAH composition and toxicity following soil bioremediation S. Simonich, Oregon State University / Dept of Chemistry and Environmental Molecular Toxicology; L. Chibwe, Oregon State University / EMT
The bioremediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs) is a common approach for chemical cleanup. The goal of the study is to isolate and identify compounds in soil, which could potentially be the cause for increased toxicity in soil post bioremediation. We developed an extraction method using pressurized liquid extraction (PLE), and fractionated the contaminants in the soil using silica solid phase extraction (SPE), by varying solvent elution polarity. The DT40 bioassay was then used to assess the toxicity of the different fractions. We observed an increase in the overall toxicity of the soil, and an increase in genotoxicity and mutagenicity in certain fractions post bioremediation.

314 Bacterial mobilization by chemotaxis as an innovative promoter for bioremediation techniques C. Jimenez Sanchez, Inst de Recursos Naturales y Agrobiol. de Sevilla / Agroquimica y Conservacion de Suelos; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiologia / Agroquimica y Conservacion del Suelo
Bioremediation techniques can be limited by a low bioavailability which represents the accessibility of the chemicals for biotransformation and toxicity to microorganisms. Even more, bacterial mobilization in soils may be restricted by several factors, including increased path lengths (tortuosity), bacterial adhesion to surfaces, and geometrical restrictions due to pores of small diameter and dead ends. We propose bacterial tactic response as a mechanism that could change the swimming behavior and hence improve bacterial mobilization in saturated porous media, thus promoting an enhanced biodegradation of pollutants. We characterized the tactic response of naphthalene-degrading Pseudomonas putida G7 to different dissolved organic matter (DOM) sources by capillary assays and by computer-assisted motion analysis. Sunflower root exudates and humic acids (HA) were used as representatives of fresh and weathered DOM at high concentrations (~50 mg C/L) and at low concentrations (~1 mg C/L). We observed a via tactic mobility in the bacterial mobilization in soils, we assessed the influence of DOM on bacterial strain deposition in porous media (sand) in well-controlled column systems. We suggest that chemotactic sensing combined with changed swimming modes could be the reason for the enhanced bacterial transport observed with sunflower root exudates. We analyzed some motility parameters like speed or ratio of changes of direction (rD) to determine the effect of exudates on swimming trajectories. Humic acids also promoted bacterial transport, but this effect was caused by other mechanism different to chemotaxis, and possibly involved physical interactions between the cell surface, the porous material and the DOM.

315 Impact of organic additives on the immobilization of heavy metals in the phytoremediation process of degraded soils A.E. Placek, A. Grobelak, Czestochowa University of Technology / Institute of Environmental Engineering; D.P. Włóka, Czestochowa University of Technology / The Faculty of Environmental Engineering and Biotechnology; A.R. Almas, National University of Singapore / Institute of Environmental Engineering
The presence of heavy metals in the natural environment, especially in areas used for agriculture, is an important issue for environmental concerns. Trance elements by plant organisms can be included in the food chain and biological circulation. A characteristic feature of heavy metals is also capability to remain in the environment for long periods of time, as well as resistance to chemical and biologica degradation. A phytoremediation process involves a variety of techniques which involves the plants for the treatment of soil from organic and inorganic contaminants. An additional advantage of phytoremediation of soils is the possibility of using waste and by-products as soil additives to support this process and at the same time to affect the recycling and disposal of a significant amount of waste substances. Soil organic additives are a source of organic matter and biogenic elements which affect a significant part of the movement of contaminants in the environment. Moreover, they have the sorption ability related to the immobilization of contaminants in soil. The aim of the research was to determine how the application of organic additives into the soil surface improves the phytoremediation process (conducted with two plant species) of soils contaminated with heavy metals. The pot experiment was 1 year carried out under controlled conditions of phytotron chamber. Soil material used in the study was collected from two sites (Poland): the area in the zone of zinc smelter influence located in (Silesia region) and also from post-mining land lignite mine dump in Belchatow. In this experiment the following plant species were used: forest species (Pinus sylvestris L.) and energy crops (Miscanthus giganteus). The amendments used for this study were different types of organic amendments: sewage sludge, composts, coal mules, lacustrine chalk to determine the degree of immobilization of pollutants in the soil. The mobility of heavy metals in the soil can be decreased by the application of organic additives such as: lacustrine chalk and compost from municipal waste compost (compost 1). Selected energy crops, because of its excellent adaptability to change habitat conditions, the possibility of gradual reclamation of degraded land and the ability to prevent the migration of heavy metals into the soil and groundwater that also enter the food chain, can be used in the remediation of soil and of sootless devastated areas, as pioneering plants.

center / Columbia Environmental Research Center; L. Kapustka, LK Consultancy; S.N. Luoma, University of California, Davis; John Muir Institute of the Environment; J.R. Rohr, University of South Florida / Department of Integrative Biology; A. Wagner, Chevron

Multiple stressors from human activities are increasingly altering the composition and integrity of our aquatic and terrestrial ecosystems. Land use change, mining impacts, increasing urbanization and climate change are already having a major impact on habitats, ecological processes and communities. The restoration of contaminated ecosystems is even more important as open spaces and aquatic resources become more limited throughout the world. The processes of preventing contamination during restoration activities and restoring contaminated ecosystems have unique issues that must be addressed including selection of ecosystem restoration tools, (to what do we restore?), restoration design (how do we restore?), restoration effectiveness (have we been successful?) and anticipating pitfalls (e.g. potential for attractive nuisance). This paper will discuss highlights of a recent joint SETAC/SEER-sponsored technical workshop on Restoration of Impaired Ecosystems. Experts in the fields of aquatic and terrestrial ecotoxicology, restoration, and related fields from both SETAC and the Society for Ecological Restoration (SERC) worked together to address issues and discuss case studies to illustrate key approaches and research needs in ecosystem restoration. The overall objective of the workshop was to define the best scientific practices available for preventive restoration to limit potential contamination and for restoration of aquatic and terrestrial ecosystems where toxics have been released. This multi-disciplinary approach and key outcomes from the workshop will be discussed.

317 The induced biodegradation of polycyclic aromatic hydrocarbons (PAHs) in cultivation of Phalaris arundinacea D. P. Wile, Czcehowa University of Technology / The Faculty of Environmental Engineering and Biotechnology; A.E. Placek, M. Kacprzak, Czcehowa University of Technology / Institute of Environmental Engineering Polycyclic aromatic hydrocarbons (PAHs) belong to the group of most commonly present in environment organic contaminants. PAHs are build from at least two aromatic rings and don't have any additional substituent s. They are hardly soluble in water, however in presence of soil soluble organic fraction, they can easily migrate to the deeper layers of soil profile. Due to the proven carcinogenic activity of some representatives of PAHS, the occurrences of these substances in agriculture soil is considered as potential risk to human health. Taking in above the maintained facts it can be stated that studies aimed to develop or evaluate new environmental friendly methods for PAHs remediation are very important. The aim of this study was to investigate the effectiveness of PAHS biodegradation in growing of Phalaris arundinacea, fertilized with sewage sludge and mineral sorbents. The study was conducted under the controlled conditions, by using the phytotrode chamber. An experiment includes three steps. The first step rely on performing complete characterization of used materials. Second step included the preparation of analytical stand and 6-month incubation. During the incubation, in week 24th the soil and leachate samples from each reactor were collected. Next, the soil samples were subjected to PAHS content analysis and the leachate samples were subjected to total carbon (TC) and total organic carbon (TOC) content analysis. Step third includes the secondary measurements of soil physical and chemical properties. Prepared experiment was consisted of 8 aerobic bioreactors. Each bioreactor was filled with following materials: 1-Soil; 2-Soil; 3-Sewage Sludge+soil; 4-soil+sewage sludge; 5-soil+mineral sorbent; 6-soil+mineral sorbent; 7-soil+sewage sludge+mineral sorbent; 8-soil+sewage sludge+mineral sorbent. On the surface of bioreactors 2, 4, 6 and 8 the seeds of Phalaris arundinacea were also been introduced. Obtained results showed that, fertilization with sewage sludge causes a temporary increase in the content of organic pollutants such as PAHs in soil. The use of sorbents as a fertilizerization material may increase the mobility of pollutants in the soil environment. Direct increase of soil sorption capacity by introducing mineral sorbents has a significant impact on the induction of biodegradation of PAHS in soil. The cultivation of crops such as Phalaris arundinacea has a positive effect on the biodegradation of PAHS in soil.

318 Strategies for cleaning up ATRAZINE-polluted soils using Microbial Electroremediating Cells (MERCs) A.D. Garay, K. Boltes Espinola, A. Esteve-Núñez, University of Alcalá Bioremediation is a cost-effective technology for treating polluted soils. However, the tolerance of microorganisms to sustain microaerobic respiration can not only reduce the microbial detoxification activity. The concept of using electrodes to overcome this metabolic limitation led recently to the concept of Microbial Electroremediating Cells MERCs (1). In contrast with standard environmental applications as sedimentary Microbial Fuel Cells (sMFC), MERCs do not aim power production but to maximize biodegradation rates around the electrode by using a never-ending electron sink. Our work demonstrates that natural populations can be effectively stimulated by MERCs in order to bioremediate a soil polluted with the herbicide atrazine (2-chloro-4-ethylamine-6-isopropyl amino-1,3,5-triazine). Residual concentration of atrazine was ca. 6-fold lower in MERC-treated soil than in MERC-free soil after 2 weeks of assay. This effective bioremediation task was confirmed by soil extraction followed by HPLC-DAD analysis. Furthermore, the effective removal of pollutants was supported by ecotoxicological analysis, based on algal growth assays, with a severe reduction in soil toxicity (ca. 30-fold) after 2 weeks of treatment leading to a non-toxic soil. Interestingly, MERC-free soil was still heavily polluted after this period, proving that MERCs can be an effective tool for removing organic pollutants from soil.

319 Exploring the potential roles of metabolites in chemical hazard assessment M.R. Viant, University of Birmingham / School of Biosciences

320 Roles for new technologies in improving the risk assessment of chemicals in consumer products S. Marshall, Unilever / Safety and Environmental Assurance Centre

321 Opportunities for omics under REACH W. De Coen, European Chemicals Agency - ECHA

322 Omics in the regulatory assessment of chemicals - challenges and chances G. Streck, European Commission, DG ENTR / Department WANA

323 Discussion - Open floor to audience J. Colbourne, University of Birmingham We will start the discussion by proposing that our speakers and audience answer the question: Can OMICS deliver solutions in time to be of practical value to REACH and WFD? We aim to understand the position of the different stakeholders regarding this problem and propose to this community a low-cost protocol, with the different assays and novel technologies, in order to achieve a good integration of this data into Risk Management. Making it more useful for the policy makers and enforcement agencies, simultaneously, allowing the industry to move at a faster pace towards responsible innovation, and presenting the scientific community with clear goals a special note on time and cost effectiveness to meet these legislative and regulatory challenges.

Periphyton ecology and ecotoxicology: how much complexity and reproducibility are required to connect both perspectives?

324 Fungicide effects on stream benthic microbial communities: influence of substratum type F. Donnadieu, F. Rossi, P. Besse-Hoggan, C. Forestier, Université Blaise Pascal; J. Arties Fungicides reaching stream ecosystems can show different capacities to adsorb onto benthic substrata, and thus, cause contrasting effects on their associated microbial communities. This study aims to evaluate the potential of tebuconazole (TBZ) adsorption onto submerged leaves and sand substrata and identify its effects on the biomass and functioning of microbial communities. An indoor stream channel experiment was designed to test TBZ effects on leaves alone, sand alone, and mixed leaves+sand substrata during five weeks. Weekly samplings evaluated TBZ effects (10.7 ± 0.3 μg L⁻¹, average exposure concentration) on microbial biomass, diversity and activity (litter breakdown rates, sporulation rates and extracellular enzyme activities) of fungi and bacteria as well as the 7-day percentage of TBZ dissipation in channels. Results showed that TBZ significantly reduced fungal biomass (17-20 % with respect to controls) but increased the bacterial biomass (34-50 %) on leaves and sand incubated alone. TBZ significantly inhibited phenol oxidase activity (36 %) in sand microbial communities but not that on leaves. Differences in TBZ effects between leaves and sand communities were not explained by differences in TBZ adsorption between substrata but more likely were related to the different biomass accrual and structure of microbial communities. Mixing (leaves+sand) substrata tended to attenuate TBZ effects on microbial communities, and this was probably explained by the greater substrate surface area available for TBZ adsorption. This hypothesis was confirmed by the greater percentage of TBZ dissipation measured in (leaves+sand) channels. However, substrata mixture did not only dilute but modified TBZ effects in sand communities [increase of fungal biomass (14 %) and hyphomycete sporulation...
rates (82 %). This result suggests that sand can eventually represent a suitable microenvironment for fungal development during TBZ contamination episodes. The present study shows the relevance of benthic substrata heterogeneity in determining the fate and effects of TBZ in agricultural streams, but also highlights the contrasting response between fungi and bacteria to cope with the fungicide.

325 Arsenic toxicity effects to microbial communities in indoor experimental channels mimicking fluvial system

A.M. Romani, University of Girona / Institut d’Ecologia Aquàtica; B. Tuulaihku, University of Girona / Environmental Science; H. Guasch, Institute of Aquatic Ecology

The aim was to evaluate the fate and effects of a long-term, environmentally realistic concentration of Arsenic (As) exposure on some of the biofilm functions in a simplified fluvial system including interaction between fish and biofilm. The presence of fish provides a high ecological realism through contribution of nutrients, since arsenic toxicity is more severe to nutrient starved peripherons. On the other hand, biofilm may also influence arsenic toxicity to fish due to adsorption, uptake and/or transformation of arsenic. Biofilms were exposed for 59 days to arsenic (120 μg/L As³, nominal concentration) under the presence of fish (Gambusia holbrooki) with a biomass of 27.95±3.7 g/m² in a recirculating system. Nutrient input from fish was calculated based on mass balance equation and compared with nutrient contents in the different compartments of the system: illuminated substrata, sediment, fish and water. Arsenic fate to the different compartments was also quantified and a set of biofilm endpoints related to community composition (algal groups), biomass growth (AFDW and Cha a) and microbial activity (phosphatase and β glucosidase) were examined. Fish had a significant contribution to phosphorus input, providing background P concentration. As also adsorbed to sediment (79.7%) leading to 40.7 μg/L As (V), as calculated daily average exposure. On the other hand, As concentration in periphyton was higher (15.9±4.8 μg/kg), supporting the use of arsenic accumulation in periphytic biofilms as biomarker of As exposure in natural systems. As exposure inhibited the growth of diatoms, moreover, considering the relative florescence of the groups of algae: diatoms dropped off significantly and the percentage of green algae increased throughout time indicating a selective pressure towards the former. Total biomass of biofilm and chlorophyll a in the sediment, were affected by As, making the biofilm heterotrophic. Phosphatase activity were significantly lower in As treatment not demanding more phosphorus in this oligotrophic system, since autotrophic biofilm growth has already inhibited, also there is direct influence of As to the bacteria in the sediment. We conclude that exposure to arsenic at environmentally realistic concentrations and conditions will directly and indirectly affect the base (primary producers) of the aquatic food chain and its respective contribution to nutrient cycling impairing the normal functioning of the ecosystem.

326 Bioavailability of mercury to natural freshwater periphyton and consequences on periphyton composition

P. Drangeit, Institut Foyer, University of Geneva / Section of Earth and Environmental Sciences Institute Foyer and Institute for Environmental Sciences; S. Le Faucheur, Section of Earth and Environmental Sciences Institute Foyer and Institute for Environmental Sciences; C. Rots, Geneva University / Institute Foyer and Environmental Sciences; V.I. Slabykova, University of Geneva / Section of Earth and Environmental Sciences Institute Foyer and Institute for Environmental Sciences

Mercury (Hg) is a global contaminant, whose most dangerous form is methylmercury (CH₃Hg) due to its biomagnification along the trophic chain. Periphyton is a complex community composed of autotroph and heterotroph organisms embedded in an abiotic matrix, which represents a pathway of Hg incorporation into the food chain. The present study aims to improve the understanding of Hg accumulation in natural biofilms along a gradient of Hg incorporation into the food chain. The present study aims to improve the understanding of Hg accumulation in natural biofilms along a gradient of Hg incorporation into the food chain. The present study aims to improve the understanding of Hg accumulation in natural biofilms along a gradient of Hg incorporation into the food chain. The present study aims to improve the understanding of Hg accumulation in natural biofilms along a gradient of Hg incorporation into the food chain. The present study aims to improve the understanding of Hg accumulation in natural biofilms along a gradient of Hg incorporation into the food chain.

327 Adaptation of periphyton communities to copper and silver in an artificially-controlled exposure

A. Autret, Irstea Antony UMR 6143 M2 CNRS / UMR MCNRS; E. Uher; I. Friedel, Irstea Antony; T. Berthe, UMR 6143 M2 CNRS; L. Fechner, Irstea Antony; F. Petit, UMR 6143 M2 CNRS

Linking urban pollution to biological effects depends on the knowledge of biotic (mechanisms). The objective of this study was to evaluate the effect of a realistic concentration of biofilm to evaluate the effect of a realistic concentration of biofilm on periphyton in ecotoxicology. After an exposure to both metals, more than 50% of the bacterial community organization was represented by two orders corresponding to Rhizobiales (23%) and Bacillus (17%). In conclusion, the bacterial periphyton communities seem to express a specific gene when exposed to silver. Quantification of silica and copper genes and the related cDNAs by qPCR will carry out the level of its occurrence and expression.

328 Complex communities exposed to multiple pollutants: using passive sampler extracts in periphyton ecology

S. Pesce, Irstea / EU EARLY; B. Tuulaihku, IRSTEA; INRS; E. Uher, Irstea; T. Berthe, UMR 6143 M2 CNRS; E. Uher; I. Friedel, Irstea Antony; T. Berthe, UMR 6143 M2 CNRS; L. Fechner, Irstea Antony; F. Petit, UMR 6143 M2 CNRS

In rivers, risk assessment requires taking into account both the complexity of contaminations (multiple substances at low concentrations), and the increasing demand for ecologically realistic biological endpoints used in toxicity testing. These issues can be tackled by coupling toxicity assessment using complex communities, such as periphyton (composed of microalgae, bacteria, fungi, protozoa, etc.), and complex contaminants like passive samplers extracts (PSE). Here we propose to review some recent progresses in ecotoxicology allowed by the joint use of PSE from POCIS (Polar Organic Chemical Integrative Samplers) and river periphyton. More specifically, we will present diverse experimental approaches, aiming: -at characterizing the "toxic potential" of waters and applying the pollution community induced tolerance –PIT– approach to pesticide mixtures, -at identifying the compounds responsible for toxicity in the mixture (effect-directed analyses -EDA), and -at increasing the environmental realism in microcosm experiments by performing chronic low dose exposure to mixtures from PSE. We were able to detect PIT in periphyton collected in rivers showing a gradient of increasing pesticide concentrations or at sites with different contamination profiles using toxicity tests with PSE from the field. From a pesticide mixture that proved to be toxic, we performed successive fractionation steps to identify the substance(s) responsible for toxicity. In the mixture we used, however, no individual fraction was found to explain the impacts of the cocktails, whereas combinations of fractions did. This highlights the need to consider the "mixture issue" in environmental risk assessment. One way to improve mixture toxicity assessment is to implement chronic exposure experiments with PSE. We successfully performed artificial channel experiments using of PSE as a complex contaminant at low doses. The environmentally relevant concentrations tested drove community changes, in their structure (biomass, composition) as well as in their functions (enzymatic activities). Altogether, these encouraging results open wide perspectives for a more realistic risk assessment, from both biological and chemical points of view.
Wildlife ecotoxicology from molecular to population effects (I)

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Residue analyses of grey partridge’s abnormal eggs: exposure to mixtures of plant protection products found in the real world
M. L. Eng, Simon Fraser University; K.G. Drouillard, University of Windsor / Great Lakes Institute for Environmental Research; S.W. Kennedy, Environment Canada / Science Technology Branch; S. Jones, Thomson Scientific / Books Acquisition; T. Williams, Simon Fraser University; K.G. Drouillard, University of Windsor / Great Lakes Institute for Environmental Research; S.W. Kennedy, Environment Canada

Unintentional effects of current plant protection products (PPPs) use on non-target animals are little documented in situ on vertebrates living in cultivated farmlands. In the present study, we evaluated the effects of plant protection products on grey partridge embryos. This declining gamebird, typical of European cereal ecosystems, is especially exposed to PPPs during its whole life-cycle and appeared as a relevant focal species. The field study was carried out on 12 sites typical of French intensively cultivated farmlands where the grey partridge is still present as wild populations. 467 females were radiotracked daily. We detected 281 clutches (> 250 eggs) over a three-year period. A total of 57,612 eggs (of hatchlings, pipped eggs as well as destroyed and intact eggs) was collected for a detailed description, some measures and further laboratory analyses. We recorded the following endpoints: clutch fate and cause of failure; clutch size; % of fertile eggs; % of dead embryos, stage of development and macroscopic anomalies; eggshell thickness. In a previous work, we determined the potential exposure of clutches to active substances during pre-laying. In the Panhandle, Rolling Plains, on the coast and some rural areas, there was a notable coincidence between partridge presence in crop fields and pesticide application on those fields (known from a survey of 142 farmers). In addition, we performed residue analyses on 80-90 eggs using screening methods including ca. 500 active substances. After a short presentation of the whole study, we will briefly describe the real data for the following endpoints: identifying suspicious anomalies in clutch or egg characteristics and quantifying them. Then we will report the results of residue analyses, focusing on the complex mixtures found in the real world and discussing the similarities and differences between potential and actual exposures. At this stage of our work, we supplemented our initial field dataset to develop in silico models to examine, and if possible to predict, the effects of simultaneous exposure to active substances and on the concentrations of their residues. This research is funded by the Pesticides – Ecohygo 2018 program of the French Ministry of the Environment.

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Are neonicotinoid insecticides associated with northern bobwhite declines in Texas?
M.A. Mora, H. Baldwin, Texas A&M University / Wildlife and Fisheries Sciences

Neonicotinoid insecticides, primarily imidacloprid, thiametoxam, clothianidin, dinotefuran, and acetamiprid have been used in Texas since 1995. In 2009, the estimated use of imidacloprid in Texas, was close to one million pounds, and it was used primarily on corn, soybeans, and wheat. The regions with the heaviest application were the Panhandle, Rolling Plains, on the coast and some rural areas, and the Lower Rio Grande Valley. Studies in the U.S. and Europe have shown that neonicotinoid insecticides could have different effects on birds which could lead to population declines. During the first two weeks of their life, quail chicks in Texas feed primarily on arthropods (>80%), including leafhoppers, flies, bees, and wasps. Insects can accumulate significant concentrations of neonicotinoids. Moreover, northern bobwhites can feed heavily on crop seeds (e.g. wheat, milo, corn, and soybean) during the fall-winter. Seeds treated with imidacloprid could possibly lead to reproductive abnormalities in birds. We compiled information on the use of neonicotinoid insecticides in Texas over the last 20+ years, using data from the USGS NAWQA database (http://water.usgs.gov/nawqa/npnps/usage/maps/). We compiled quail population data from Texas using the Texas Parks and Wildlife Department roadside survey data collected over the same time period. We correlated quail population trends with neonicotinoid insecticide use in Texas to establish possible cause-effect linkages. The results indicate a significant negative correlation between neonicotinoid insecticide use in Texas and quail population declines. We are currently conducting GIS analysis to more effectively determine the relationship between quail population changes and heavy neonicotinoid use in important agricultural areas in Texas. Additionally, data on tissues and crop contents of bobwhite quail collected over all the state will be examined and correlated with areas of heavy use of neonicotinoids in Texas.

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Identity of key amino acids in the AHR1 ligand-binding domain predicts avian sensitivity to dioxin like compounds: in vivo verification in European Starlings
M.L. Eng, Simon Fraser University; J.E. Elliott, Environment Canada / Science Technology Branch; S. Jones, Thomson Scientific / Books Acquisition; T. Williams, Simon Fraser University; K.G. Drouillard, University of Windsor / Great Lakes Institute for Environmental Research; S.W. Kennedy, Environment Canada

Avian species are highly variable in their sensitivity of to the embryotoxic effects of dioxin. The objective of this study was to test that prediction in vivo. To do this, we used egg injections in field nesting starlings with 3,3',4,4',5-pentachlorobiphenyl (PCB-126), a dioxin-like PCB. Eggs were dosed with either the vehicle control or one of five doses (1.4, 7.1, 15.9, 32.1, 52.9 ng PCB-126/g egg). Eggs were incubated by parents until pipping, and then placed on an incubator to monitor hatching so that individual hatching could be attributed to specific eggs. Hatching success of the vehicle treated eggs was equivalent to unmanipulated starling eggs (94%). There was a dose-dependent increase in embryo mortality, and the LD50 (95% CI) was 5.61 (2.33-9.08) ng/g. Hepatic CYP1A4/5 mRNA expression in hatchlings also increased in a dose-dependent manner, with CYP1A4 being more induced than CYP1A5. There was no effect of dose on morphological measures, and we did not detect any overt malformations. These results indicate that other than the chicken, the European starling is the most sensitive species to the effects of PCB-126 on avian embryo mortality reported to date, which supports the prediction of relative sensitivity to DLCs based on amino acid sequence of the ARH1.

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Wetlands receiving effluents of wastewater treatment plants: an ecological trap for waterbirds
J. Lopez Perez, Instituto de Investigacion en Recursos Cinegeticos / Unidad de Ecologia y Ciencia Animal; J. Felia, Instituto de Investigacion en Recursos Cinegeticos; P.R. Camarero, UCLMSCIC; R. Mateo, U.C.L.M.-C.S.I.C. / Instituto de Investigacion en Recursos Cinegeticos

The effect of other pollutants being accumulated in Navaseca lagoon like heavy metals or other organic pollutants or even by the presence of avian pathogens in waterbirds from a wetland that receives the effluent of a wastewater treatment plant (Navaseca Lagoon) and how these pollutants affect their levels of plasma antioxidants compared with birds from non-polluted sites (Tablas de Daniel National Park), Blood samples were collected of total number of 99 common moorhens (Galina chloropus) to determine plasma levels of vitamins (retinol and t-tocopherol) and carotenoids (lutein) and blood concentrations of OC pesticides and PCBs. The most prevalent OCs in blood of common moorhen were p,p'-DDE, p,p'-DDD, HCB and 2PCBs. Moorhens showed higher levels of PCBs in Navaseca than in Tablas de Daniel National Park and a similar trend was observed for the other persistent pollutants. The levels of plasma retinol were higher in Tablas de Daniel than in Navaseca Lagoon and a similar non-significant trend was observed for t-tocopherol and lutein. Differences observed here in retinol levels could be associated with the exposure to persistent pollutants, in especial to PCBs. However, the low negative correlation found between retinol and PCBs may be explained by the contribution from other pollutants being accumulated in Navaseca lagoon like heavy metals or other organic pollutants or even by the presence of avian pathogens in the area. Our results indicate clear differences in the accumulation of PCBs and the retinol levels between the two study areas. Retinol has important roles in functions involved in the maintenance of the health status of animals. Birds attracted to these highly productive eutrophic wetlands that receive effluents from wastewater treatment plants may suffer negative impacts on their health due to the accumulation of persistent pollutants and the consequent retinol status unbalance.

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Pb shot ingestion, oxidative stress and carotenoid-based coloration in waterbirds from the Ebro delta, NE Spain
N. Valverde Coll, Instituto de Investigacion en Recursos Cinegeticos / Wildlife Toxicology; F. Mougeot, EEEA; M.E. Ortiz Santaliestra, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; A. Lopez-Antia, Instituto de Investigacion en Recursos Cinegeticos (IREC); J. Rodriguez-Estival, University of Calgary / Ecosystem and Public Health; R. Mateo, U.C.L.M.-C.S.I.C. / Instituto de Investigacion en Recursos Cinegeticos

The ingestion of lead (Pb) shot constitutes the main cause of Pb poisoning in avifauna, but less is known about the sublethal effects and their consequences at a population level. We studied the relationship between Pb shot ingestion, oxidative stress and coloration in mallards from the Ebro delta. A total of 109 mallards were caught between 2008 and 2009 and the same samples were treated to determine Pb concentrations and δ-ALAD activity (9). Eggs were dosed with either the vehicle control or one of five doses (9). There was a dose-dependent increase in embryo mortality, and the LD50 (95% CI) was 5.61 (2.33-9.08) ng/g. Hepatic CYP1A4/5 mRNA expression in hatchlings also increased in a dose-dependent manner, with CYP1A4 being more induced than CYP1A5. There was no effect of dose on morphological measures, and we did not detect any overt malformations. These results indicate that other than the chicken, the European starling is the most sensitive species to the effects of PCB-126 on avian embryo mortality reported to date, which supports the prediction of relative sensitivity to DLCs based on amino acid sequence of the ARH1.
levels were positively related to blood Pb concentration. A significant interaction between sex and blood Pb levels explained the variation in retinol (p = 0.033), GSH (p = 0.079) and GSHx (p = 0.017) levels, with negative relationships in females and positive relationships in males. In females, leg orange ness did not vary with blood Pb levels. In males, leg orange ness (p = 0.010) and beak yellowness (p = 0.008) negatively correlated with blood Pb levels. Beak yellowness positively correlated with blood Pb concentration (β=0.03 per mg l⁻¹ Pb), however, and the association between blood Pb and coloration remained when controlling for these parameters (all p < 0.017). Although blood Pb levels decreased after the ban on Pb ammunition, Pb exposure still occurred in 2012, and affected several oxidative stress indicators and coloration in males, which is known to influence reproductive investment in mallards. Exposed females may have compensated Pb effects by investing antioxidants to counter oxidative stress, without compromising coloration. Males may have mobilized antioxidants to counter Pb-induced oxidative stress at expenses of coloration, reflecting the carotenoid-antioxidant allocation trade-off between the expression of sexual signals and the oxidative stress-sensitive functions.

334 Environmental and anthropogenic stressors and impacts on gulls S. Lacorte, A. Bertolero, IDAEA-CSIC / Environmental Chemistry; E. Abad, CSIC / Dioxin Laboratory Environmental Chemistry Department; C. Porte, CSIC - IQAB / Environmental Chemistry; S. Diez, CSIC-IDAEA / Environmental Chemistry; F. Santos, University of Barcelona / Analytical Chemistry Nourishes, many habitats are very likely of importance in terms of both the environmental conditions and the availability of food. Therefore, understanding the relative impact of environmental and environmental pressures which can affect the wellbeing of many species. This study is focused on the effects of environmental and anthropogenic stressors on gulls, considering effects that span from biochemical to population level. The species studied are the yellow-legged gull (Larus michahellis, YLG) and the Audouin’s gull (Larus audouinii, AG). YLG is a common, large and oportunistic gull, with an omnivorous diet but often scavenges on refuse tips. On the other hand, AG is a medium size gull, endemic of the Mediterranean area, categorized as Near Threatened species on the IUCN 2012 Red List (IUCN, 2012). AG is a piscivorous species, considered a specialist in the capture of chupeformes (pelagic fishes), but they can also feed on fishery discards and terrestrial preys. Whereas YLG is widely spread over the Iberian Peninsula, AG is concentrated in the Ebro Delta and Chafarinas colonies. In Spain, gull colonies are generally settled in National, Hunting Refugees and Natural Parks, areas with high levels of conservation and protection. However, gulls, and indirectly other species, are affected by multiple stressors that can induce effects at individual or population level. We have first determined the pressures in the National Park of Atlantic Islands, in Chafarinas Islands and in the Ebro delta Natural Park. The species studied were YLG and in the Ebro delta, we also studied AG. In each colony, we have collected gull eggs and eventually blood. The array of parameters studied were: Characterization of Persistent Organic Pollutants (POPs) in gull eggs; bioaccumulation; maternal transfer of contaminants; neurotoxicity; endocrine disruption; eggshell thinning; population effects. Gulls are affected by the human impact and by the environmental stressors of the gull. It is important to study and assess these effects, in order to assess biological effects the two conventional bioassays FET and EROD were applied. In order to assess the bioavailability and the effects of contaminants, we used the two conventional bioassays FET and EROD. The method was rendered high sensitivity of the method. Equilibrium passive sampling as a tool to study hydrophobic organic chemicals in the sediment pore water of the German Bight (North Sea) and Wadden Sea N.C. Niehus, Hamburg University of Applied Sciences (HAW) / Department of Environmental Engineering; B. Brockmeyer, German Federal Maritime and Hydrographic Agency (BSH); T. Seiler, RWTH Aachen University / Ecosystem Research; G. Witt, HAW Hamburg / Department of Environmental Engineering Hydrophobic organic chemicals (HOCs) like polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are of high interest in terms of today’s sediment risk assessment and particularly bioavailability, bioconcentration and baseline toxicity. Passive sampling allows to determine freely dissolved concentrations and actually describe the transfer from sediment into the water phase. Ex-situ solid phase micro extraction (SPME) as a passive sampling tool was applied to measure the concentration of hydrophobic organic chemicals (C_{bio}) and chemical activities (α) of PAHs and PCBs in sediment samples from the North Sea and Wadden Sea. In order to assess biological effects the two conventional bioassays FET and EROD assay were conducted for investigating AYE extracts of the North Sea samples. Additionally a passive dosing version of the FET using silicone O-Rings loaded with single PAHs was performed. The composition of C_{bio} among the North Sea sampling stations was similar, and as a consequence also the chemical activities. Freely dissolved concentrations of PAHs and PCBs in North Sea sediments were rather low and ranged between 0.79 10⁻³ and 1.34 ng/g. Pyrene showed the highest concentrations in all samples. This suggests a special burden of pyrene, since it is less soluble than phenanthrene or anthracene, yet there is a higher concentration in the pore water. Compositions of C_{bio} in Wadden Sea sediments were different from the North Sea because PCB concentrations were higher, especially PCB 153. AYE extracts in the bioassays gave higher effects for samples from near the Elbe estuary, while extracts of sediments from the open sea showed no effects. The findings of the present work are compatible with the higher concentrations near the Elbe estuary. Using silicone O-Rings in the FET as passive dosing devices was easy viable and showed a good reproducibility. Naphthalene, phenanthrene, anthracene and fluorene exhibited high mortality at saturation level while fluoranthene showed low and benzo[a]pyrene no effects. In order to investigate PAH mixtures in realistic compositions, a passive dosing FET using recreated North Sea / Wadden Sea pore water is currently conducted.

338 Field Assessment of Natural Attenuation Processes Using Passive Sampling Methods
Regardless of the dosing mode, 4 PBDEs, HCB and DDT as identified in human silicone breast implant isomers (4 compared to conventional solvent as sorptive sink for lipophilic hormones produced partitioning for human health by interfering with the endocrine system. A new University of Denmark / National Food Institute Environmental Sciences; P. Mayer, Technical University of Denmark / Department D. Gilbert passive dosing from silicone generating scientifically accurate water the samplers were experiencing and measuring similar C sample concentrations were also measured (i.e., grab samples), passive investigation was to evaluate the use of passive sampling for measuring water contaminants (HOCs). Historically acquiring the freely dissolved concentration free contaminants to understand the complete exposure of aquatic organisms to hydrophobic organic concentrations (C) of HOCs was challenging. In recent years, passive sampling has been demonstrated to be an effective tool for determining Cwater in the water column and in sediment interstitial waters. Currently, there is an effort underway encouraging remedial project managers (RPMs) at contaminated sites to use passive sampling to collect Cwater data in order to improve site assessments. The objective of this intercomparison study was to assess the use of passive sampling for measuring water column Cwater for several HOCs at three U.S. EPA Superfund sites. Sites investigated included New Bedford Harbor, Palos Verdes Shelf and Naval Station Newport and the passive samplers evaluated were polyethylene (PE), solid phase microextraction (SPME), semi-permeable membrane devices (SPMD) and polyoxymethylene (POM). In general, the different passive samplers demonstrated good agreement with Cwater values varying by a factor of two to three. This level of agreement was demonstrated, in particular, at Palos Verdes Shelf where elevated Cwater determined by different types of passive samplers deployed in studies over several years varied by less than a factor of two (i.e., 320 to 460 pg/L for sum of polychlorinated biphenyls, PCBs). Further, at New Bedford Harbor, where conventional water sample concentrations were also measured (i.e., grab samples), passive sampler-based Cwater values were only a factor of two. These findings suggest that all of the samplers were experiencing and measuring similar Cwater during their respective deployments. This evaluation demonstrates the utility of passive sampling for generating scientifically accurate water column Cwater which is critical for making informed environmental management decisions at contaminated sediment sites.

340 Potential of human POP levels for endocrine disruption - applying in-vitro passive dosing from silicone

D. Gilbert, Aarhus University, Science and Technology Faculty / Department of Environmental Sciences; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; A. Vangued, M. Pedersen, DTU Technical University of Denmark / National Food Institute

Persistent organic pollutants (POPs) accumulated in human tissue may pose a risk for human health by interfering with the endocrine system. A new partitioning-controlled dosing approach from silicone was applied in the in-vitro H295 steroidogenesis assay to test whether POPs in a mixture composition and at concentrations that can interfere with steroidogenesis can act as partitioning agents for the test chemicals but at the same time functioned as sorptive sink for lipophilic hormones produced by the cells. The new method was compared to conventional solvent-dosing. A technical mixture of 4-nonylphenol isomers (4-NP) was used for method development, and finally a mixture of PCBs, PBDEs, HCB and DDT as identified in human silicone breast implants was tested. Regardless of the dosing mode, 4-NP altered the hormone production in a qualitatively similar dose-dependent manner. With the concentration of freely dissolved molecules in the exposure medium as dose metrics as a measure for the effective concentration, dose-response curves were moved toward lower concentrations by a factor of approx. ten relative to nominal concentrations in the medium. The POP-mixture, when dosed from silicone, increased the production of progesterogens and androgens with exception for testosterone, but not when co-sovent dosing was applied. The new silicone-based dosing approach was successfully established and allowed linking human silicone concentrations of POPs to altered hormone production in the human cell line H295.

341 Linking transcriptome response with mercury accumulation in the microalgae Chlamydomonas reinhardtii

R. Beauchais-Flueck, Institute Forel Earth and Environmental Sciences; V.I. Slaveykov, University of Geneva / Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences; C. Cosio, Geneva University / Institute Forel Earth and Environmental Sciences

Mercury (Hg) pollution is a continuing toxic issue for freshwater ecosystems producing understanding of and effects of both inorganic (IHg) and methyl (MeHg) Hg forms in key organisms is essential to predict the risk they could represent for the aquatic environment. This study aims at investigating the link between internalized Hg concentrations and the expression of genes, obtained by high-throughput sequencing (RNA-Seq), in the microalgae Chlamydomonas reinhardtii. Algal cells were exposed two hours in a simplified artificial medium spiked with series of inorganic Hg concentrations (1 x 10^-6, 1 x 10^-5 and 1 x 10^-4 M IHg) or methyl Hg concentrations (5 x 10^-11, 5 x 10^-10, 5 x 10^-9 and 5 x 10^-8 M MeHg). At the end of the exposure, extracellular loosely bound Hg was distinguished from the intracellular Hg by rinsing cells with 1 mM EDTA + 1 mM cysteine, which allowed to measure intracellularly bioaccumulated total Hg or MeHg concentrations by the automatic mercury analyzer AMA254 or the MERX-MeHg System, respectively. Moreover, oxidative stress was assessed using the biomarker CellRox® and photosynthesis efficiency was evaluated by Fast Repetition Rate Fluorometry and chlorophyll fluorescence measurement. Subsamples of algae from each biological triplicate were additionally harvested by centrifugation and frozen in liquid nitrogen, before RNA extraction and library preparation. Libraries were then sequenced on Illumina NextSeq 500. Although Chlamydomonas accumulated IHg and MeHg in a dose-dependent relationship (up to 2.66 ± 0.17 amol/cell and up to 0.59 ± 0.08 amol/cell intracellular concentrations, for IHg or MeHg exposed algae respectively), no oxidative stress nor photosynthesis deficiency were observed. However, differential gene expression analysis demonstrated that exposure to IHg induced up-regulation of genes involved in the amino acids metabolism and down-regulation of genes involved in ion transport. Exposure to MeHg affected the cell cycle, with an up-regulation of microtubules and cell division linked genes, and induced divalent metal ion transport down-regulation. Several interesting genes were selected and will further be proposed as potential candidates for use as IHg and/or MeHg exposure biomarkers. This study finally showed that using transcriptomics in relation to metal bioavailability enables to detect toxic effects and connected concentrations, which could contribute interestingly in Hg environmental risk assessment.

342 Biogeochemical Factors Regulating Mercury Cycling in a Tidal Estuary and Implications for Remedy Decision-Making: Berry’s Creek Study Area, New Jersey

R. A. Berry, Anchor QEA; D. Vlassopoulos, Anchor QEA LLC; D. Glaser, Anchor QEA; P. O’Day, University of California Merced; S.S. Brown, The Dow Chemical Company / Department of Toxicology

Berry’s Creek is a tidal tributary of the Hackensack River in the northeastern United States with a long history of human impacts. PCBs and mercury (Hg) have been identified as contaminants of interest. The Berry’s Creek watershed is currently the focus of investigation by a group of private companies and public agencies under the purview of the USEPA. As part of this process, a conceptual biogeochemical model was developed to provide a basis for understanding Hg bioavailability and informing decisions on remedial action. The conceptual framework accounts for key aspects of Hg partitioning (aqueous speciation, precipitation of inorganic sulfide, binding to organic matter), and methylmercury (MeHg) formation, demethylation and partitioning to sediment (net MeHg accumulation). A thermodynamic-kinetic reaction path model was constructed to simulate/explore relationships between total Hg and MeHg concentrations over a range of sediment conditions. Theoretical upper limiting values for MeHg concentrations in sediment are predicted by the model as a function of total Hg load and inorganic Hg partitioning mechanisms in sediment (sorption vs. solubility). Net MeHg accumulation less than the theoretical maximum is due to biogeochemical redox processes that suppress Hg methylation and/or enhance MeHg demethylation. The general nature of our model is empirically confirmed by comparison of model predicted limits with data for sediments covering a wide range of Hg concentrations and environments across the study area. Measured MeHg levels are commonly up to 2 to 3 orders of magnitude lower than upper limiting values and correlate with oxic or suboxic redox regimes characterized by nitrate and manganese(IV) reduction, not sulfate reduction. Sequential extraction analysis and bulk and micro-focused X-ray absorption near-edge spectroscopy (XANES) were also used to directly determine Hg availability and solid-phase Hg and sulfur (S) speciation in
Berry’s Creek sediments. Metacinnabar was identified by bulk XANES as a significant component. S micro-XANES showed mixtures of reduced and oxidized S species. Sequential extraction analyses confirm that Hg sequestered in sulfide phases is less available for methylation. Our biogeochemical model provides a quantitative theoretically grounded framework for interpreting relationships between total Hg and MeHg levels across the study area with important implications for risk assessment and remedial approaches.

343 Assessing forty years of mercury measurements in Mediterranean Sea biota
S. Cinnirella, CNRInstitute of Atmospheric Pollution Research; P. Nicola, Institute of Atmospheric Pollution Research; D.C. Evers, D. Buck, Biodiversity Research Institute

Along the forty-year study mercury measurement in Mediterranean Sea biota, a number of techniques have been developed including more precise instruments for many assessments evaluating the impact of mercury pollution on human health and environment. Most of the study do not produced structured databases, which can improve regional assessments. To this end a wide literature survey was conducted, recognizing the necessity to document mercury concentrations in marine biota. Data were collected and archived data into a structured database under the Global Mercury Observation System (GMOS) Spatial Data Infrastructure (www.gmos.eu/sdi). The analysis of such a large database show that it is challenging to assess the biomagnification of methylmercury because there is a lack of information on measurements at different trophic levels through the food web.

344 Coping with mercury in a warmer ocean: tissue partitioning, speciation, and ecophysiological implications in seabass (Dicentrarchus labrax) ar
A. Manvula*, IPMA, IP. / Division of Aquaculture and Seafood Upgrading; P. Anacleto, Portuguese Institute for the Sea and Atmosphere / Division of Aquaculture and Upgrading; A. Lopes, Faculdade de Engenharia - Universidade do Porto / LEPAEB Laboratório de Engenharia de Processos Ambiente Biotecnologia e Energia; T. Repolho, R. Rosa, Faculdade de Ciências da Universidade de Lisboa / MARE Marine and Environmental Sciences Centre; P. Pousão, IPMA Portuguese Institute for the Sea and Atmosphere; M. Diniz, Centre for Fine Chemistry and Biotechnology (UNL) Lisbon; A.M. Marques, Portuguese Institute for the Sea and Atmosphere / Division of Aquaculture and Upgrading Anthropicogenes activities have had great environmental challenges: remarkable chemical contamination and dramatic climate change. Both factors strongly affect marine ecosystems and are expected to worsen in the future, threatening consumers’ health and marine species’ welfare and survival. It is unknown how marine ecosystems will share the environmental stressors and chemical contamination. Among chemical contaminants, methylmercury (MeHg) is of special interest, because it is highly neurotoxic, bioaccumulative and biomagnifiable throughout the aquatic food chain, and its presence in seafood is still not regulated. Thus, this contaminant is also of particular interest from the public health point of view, as the consumption of seafood is one of the main sources of human exposure to MeHg. The present work aimed to investigate, for the first time, the effect of global warming on tissue partitioning and speciation of Hg, using seabass (Dicentrarchus labrax) as model organism, and assess the organism ecophysiological consequences. Data clearly showed that temperature strongly influences Hg bioaccumulation, speciation and tissue partitioning. Moreover, synergistic effects of ocean warming and Hg exposure revealed widespread implications for the mercury uptake, and significantly impact in the form of MeHg, was positively correlated with the metabolic rates. Considering the deleterious impacts of climate change and chemical contamination, the present work suggests great biological challenges to marine populations in the NE Atlantic coastal ecosystems in the future, as well as increased risk of human exposure to MeHg through the consumption of seafood.

345 Declining Mercury in Landlocked Char in High Arctic Lakes: Response to global emissions or impacts of climate change?
D.C. Muij, Environment Canada / Aquatic Contaminants Research Division; G. Karpinski, Institute of Marine Biological Research; X. Wang, Environment Canada; K. Hudelson, University of North Texas / graduate student; P.E. Dreveny, Inrs-Ete, Universite Du Quebec / INRSITE

As the only top predators in most high latitude Arctic lakes, landlocked char are good indicators of changes in inputs of mercury (Hg) and bioaccumulation of methyl Hg. Landlocked Arctic char collected have been annually for the past 10 or more years from near the Resolute Bay on Cornwallis Island (Amituk, Char, North, Small, and Resolute) at 75 deg N. Data were collected and archived data into a structured database under the Global Mercury Observation System (GMOS). The analysis of such a large database showed that it is challenging to assess the biomagnification of methylmercury because there is a lack of information on measurements at different trophic levels through the food web.

346 NICOLE Mercury Working Group: Risk-based Management of Mercury-Impacted Industrial Sites
O. Phipps, ERM / Contaminated Site Management; R. Jacquet, Solvay SA / European Rehabilitation Management Group

The Network for Industrially Contaminated Land in Europe (NICOLE) Mercury Working Group (www.HgSafety.net) in early 2015 a guidance booklet to share case studies and best practice related the characterisation and risk-based management of industrial sites impacted by Mercury (Hg). NICOLE prepared the guidance booklet in response to recent regulatory developments to share up-to-date research, case studies and best practice for the characterisation and risk-based management of industrial sites impacted by Mercury. The booklet draws upon over 50 published references, including governmental guidelines and research papers, together with technical papers presented at the Mercury Technical Workshop organised by NICOLE in Brussels (December 2012) and the ImaHg publication produced by the SNOWMAN Network. In addition, case studies are included in the booklet prepared by industrial operators and service providers. An overview of recent legislative developments and a summary of Hg chemistry and fate & transport are provided, followed by the presentation of best practice for the risk-based management of Hg-impacted industrial sites, including characterisation, risk assessment, remediation and long-term risk management. The booklet concludes with Best Practice recommendations, initially for site characterisation and risk assessment and then for remediation and long-term site management. The presentation will show how the Roadmap project developed studies and experience (good and bad) in the characterisation and risk-based remediation/management of Hg-impacted industrial sites. Particular focus will be given to the cost-benefit assessment of waste disposal facilities, in-situ and ex-situ stabilisation techniques and soil sorting and washing approaches. Key uncertainties and pitfalls to be aware of during site characterisation and remediation works will also be shared. The presentation will highlight the key Hg Safety network case studies and mitigation measures during the characterisation and remediation of Hg-impacted sites.

Towards a reliable environmental impact assessment of personal care products

347 Exposure to environmental contaminants: Personal Care Products bioaccumulation in human placental tissue
J. Valle-Sistac, IDAEA-CSIC / Environmental Chemistry; D. Molins-Delgado, Environmental Chemistry; M. Diaz, L. Buitier, Hospital Sant Joan de Déu / Endocrinology; S. Diaz-Cruz, IDAEA-CSIC / Environmental Chemistry; D. Barcelo, IQAC-CSIC / Institute for Environmental Assessment and Research

Last decade, ingredients in personal care products (PCPs) have been described as chemicals of increasing environmental concern because of their toxicity, persistence, bioaccumulation, and ubiquity in the environment. PCPs are produced in large quantities (estimated as millions of tons per year. Parabens (PB) and UV filters (UV-F) are chemicals widely used as preservatives and sun blocking agents, respectively by personal care products, pharmaceutical and food industries. Many studies show the tendency of UV-F to bioaccumulate in living organisms due to their lipophilicity and stability versus biotic degradation [1, 2]. The ubiquity of these compounds in the environment and even human fluids [3-5] makes necessary further studies to fill the current knowledge gap. This study aims to provide evidence of the bioaccumulation of the environmental contaminants UV-F and PB in humans. The work describes the development, validation and application of an analytical method for multiclass determination of selected UV-F and PB based on high-performance liquid chromatography-quadrupole-linear ion trap-tandem mass spectrometry (HPLC-QqIT-MS/MS) in women placental tissue. Target compounds were selected by their endocrine disruption activity and frequency of detection in the environment, i.e. benzophenone-1 (BP1), benzophenone-2 (BP2), benzophenone-3 (BP3), benzophenone-4 (BP4), 4-hydroxybenzophenone (4HB), methylparaben (MPB), ethylparaben (EPB), propylparaben (PPB), butylparaben
polishing ones for triclosan and some fragrances (> 70 %). A release was observed (rainfall). In secondary sy...

In recent years, the issue of personal care products (PCPs) in wastewater has become a major concern as these substances can provide adverse effects to both human health and environment. Common wastewater treatments are not designed to deal with these compounds and data of their removal efficiencies are still scarce. This study is based on about 40 peer reviewed papers referring to removal of cosmetic ingredients from wastewater treatment. The models used were developed by these new specific QSARs, by using alkyl ether sulfates (AES, widely used in personal care products such as shampoos and lotions) (5 to 70 μg L\(^{-1}\)) and benzenes, followed by alkyl ether sulfates (AES, widely used in personal care products such as shampoos and lotions) (5 to 70 μg L\(^{-1}\)) and the more hydrophilic alcohol ether oxethoxylates (AOEs, used in household and industrial detergents) (from 5 to 50 μg L\(^{-1}\)). Regarding groundwater, the trends in terms of surfactant concentrations were similar to that previously observed for surface water samples. Therefore, the lowest values were observed during dry weather conditions (<37.4 μg L\(^{-1}\)), whereas the contamination of groundwater became more intense in December 2010 as the heavy rain caused the river to overflow and land was covered with water during several days before the sampling took place. Regarding other PCPs, 7 out of 40 were detected in surface waters: the most abundant fragrances, OTNE and galaxolide (up to 6540 and 2748 ng L\(^{-1}\)), respectively, followed by tonalide (up to 259 ng L\(^{-1}\)).

Triclosan, an antibiotic commonly used in personal care products and in some household ingredients, was predominant over the rest of target compounds (up to 1285 ng L\(^{-1}\)), followed by benzophenone 3 and EHMC (both sunscreen products) (<500 ng L\(^{-1}\)).

### 348 Flooding effect on the distribution of personal care products in detrital aquifers.

**Case study: Guadalete River basin (SW, Spain)**

C. Cordi, N. Torres-Fuentes, University of Cadiz / PhysicalChemistry; M.G. Pinto-Herrera, University of Cadiz / C. Candel, Polytechnic University of Catalonia; M. Paniz, University of Cadiz; E. Gonzalez-Mazo, University of Cadiz / Physical Chemistry; P. Lara-Martín, University of Cadiz

**Presence and distribution of personal care products (PCPs) and other emerging contaminants in aquifers are controlled by changes in their inputs. In this sense, storm events are of major importance in areas subjected to water scarcity, especially if they occur on a consecutive basis. In the Guadalete basin, flooding events caused by intense rainfalls and intense rainstorms at dry periods (May 2007) and typhoons (October 2007) resulted in the transport of PCPs and other emerging contaminants by runoff.**

### 349 Constructed wetlands: a barrier against discharge of personal care products in water environment

P. Verlicchi, University of Ferrara / Engineering; E. Zamblerto, University of Ferrara / Engineering

In recent years, the issue of personal care products (PCPs) in wastewater has become a major concern as these substances can provide adverse effects to both human health and environment. Common wastewater treatments are not designed to deal with these compounds and data of their removal efficiencies are still scarce. Concreted wetlands (CWs) are very commonly used for treating domestic wastewater across the world. This study assessed the impact of CW effluents on the aquatic environment, focusing on the application of a wide spectrum of PCPs in the influent and effluent of different types of CWs, including free water surface systems, as well as horizontal and vertical subsurface flow beds. CWs acting as primary, secondary or tertiary steps as well as CWs in hybrid systems were analysed and the corresponding removal efficiencies discussed. This study is based on about 40 peer reviewed papers referring to investigations carried out in Europe (64 %), America (38 %) and Asia (8 %).

Included PCPs belong to the following classes: antioxidants, antiseptics, deodorants, flame retardants, insect repellents, plasticizers, sun screen products, fragrances and surfactants. PCP removal mechanisms (sorption, biodegradation, photodegradation and volatilization) were investigated as well as the influence on the removal efficiencies of the main CW design parameters (i.e. bed depth, filling material, presence of vegetation, operational hydraulic load and retention time, feeding mode, redox conditions) and environmental conditions (temperature, rainfall). In secondary systems the highest average removal values were found for the hydroxamic acid (99 %) and methyl dihydrojasmonate (MDHI, 95 %), in polishing ones for triclosan and some fragrances (>70 %). A release was observed for a group of surfactants for which the formation, due to the degradation of others, is faster than their removal. An analysis of the effluent concentrations could provide, together with ecotoxological data, an estimation of the impact that these compounds could have on the environment. CWs might represent a reliable and feasible treatment which is able to control and reduce the spread of PCPs in the aquatic environment (with limited operational costs) thanks to the different stages in which they are removed from these systems and their buffer capacity. These systems could be adequate for the treatment of wastewater in small communities that are not collected to the sewage network.

### 350 QSAR Modeling and further priority setting of Personal Care Products

S. Casazza, University of Insubria / DDiTA; F. Mazzetta, University of Insubria / QSAR Res Unit Environ Chem Ecotox Department of Theoretical and Applied Sciences DDiTA; P. Gramatica, University of Insubria / DDiTA

**Nowadays, Personal Care Products (PCPs) and their ingredients are widely and daily used all over the world, often to improve human life and quality of products, and therefore to give benefits in everyday life. During the last years, PCPs became toxic. The predictions were confirmed by Principal Component Analysis (PCA), used to detect PCPs in quantifiable amounts in water compartments and thus may harm aquatic life; some of these products contain endocrine disrupting compounds, and many of their other adverse effects are unknown. Additionally, data on persistence and toxicity and the lack of overall environmental behavior needs to be investigated. PCPs show highly heterogeneous chemical structures and different properties. Due to this high variety and to the big number of end-points that should be studied, it is very important to have tools able to quickly highlight the most dangerous compounds, focusing the experiments only on the prioritized chemicals. The determination of all the requested properties is a long and difficult task. In silico tools, like Quantitative Structure-Relationship (QSAR) models, can predict missing data for the unknown activities and properties necessary to prioritize existing compounds or not yet synthesized substances. In this study, we have modeled with QSAR different properties of PCPs, useful for their environmental characterization and to evaluate their impact on biota and potential PBT (persistence, bioaccumulation, toxicity) behavior. More than 500 chemicals, such as flavor and fragrance agents, hair dyes ingredients, phthalates and sunscreen agents have been modeled and screened for some environmental physico-chemical properties (e.g. Koc, vapor pressure and water solubility), acute toxicity on Pimephales promelas and cumulative BBT behavior. New local QSARs for PCPs have been developed and the models show good parameters regarding internal and external validation. The proposed screening has been performed by these new specific QSARs, by global models already included in the QSARINS software and by different web tools. The results are a valid help for the understanding of the behavior of hundreds of PCPs, assessing their environmental impact, and for the reduction of experimental tests. A priority list containing the potentially most hazardous PCPs ingredients is proposed.**

### 351 Screening Level Environmental Risk Assessment (ERA) of Cosmetic Ingredients in the USA

J. Davies, Personal Care Products Council / Science

The Personal Care Products Council’s (PCPC) environmental program recently began the development of a new ERA framework for cosmetic ingredients in North America aligned with the ingredient groupings defined by ECOSSAR models from within the USEPA’s EPISuite toolbox. Overall tonnages of cosmetic usage were estimated using a "market forensics" approach (DeLeo et al., 2011) and industry survey data. From these combined approaches, an MST (maximum sustainable tonnage) value was derived for each cosmetic ingredient. This represents the total concentration of an ingredient which can be present in the environment without causing an adverse ecological effect. This value was then compared to total usage (of an individual ingredient) so that a risk ratio (similar to a traditional PEC: PNEC) could be calculated and assessed on an ingredient by ingredient basis. Other screening methods were developed for the initial evaluation of other ingredient types, such as polymers. Once a screening level assessment has been conducted ("tier I" of the framework) certain ingredients will then enter a second tier of assessment ("tier II"). In addition, a number of other prioritization schemes were developed, such as use of VCRP (Voluntary Cosmetic Reporting Program) data.
Natural substances enjoy great public acceptance in personal care products, as well as in medicine. Many of them are complex mixtures of compounds belonging to various chemical substance classes, and some constituents are known for their potent physiological effects. As the discharge of personal care products is mainly via WWTP into the aquatic environment, the question is whether they exert negative effects on aquatic organisms. Hazard assessment via classification and labelling is a straightforward first approach to answer this question. It is also a precious step in the prioritization process for risk assessment. Natural substances appearing in the “inventory … of ingredients employed in cosmetic products” (INCI list) were compared with the entries in the C&L inventory, which is the official European database of classification and labelling information on notified and registered substances received from manufacturers and importers according to the CLP-Regulation EC 1907/2006. Out of around 1400 natural substances in the INCI list, 655 appear in the C&L inventory with 369 natural substances being classified as hazardous substances. 185 substances are classified due to their negative effects on the aquatic environment with 184 for long lasting effects. (H400 is based on data for acute toxicity only, whereas H410, H411, H412 and H413 are based on data on chronic toxic effects, on degradability or on the potential for bioaccumulation.) Even if production volumes are not known, the number of notifiers might be used as an indication in the prioritization process. Answers to the following questions are addressed: Which of the relevant natural substances of the INCI list are labelled and classified as dangerous to the environment? Which are relevant chemical constituents and which are their functions in personal care products? Many natural substances used in personal care products are naturally toxic and demand a prudent handling and sensible regulations.

Leaving the comfort zone. The challenge of measuring nanoparticles in complex matrices

353 Single Particle ICP-MS (SP-ICP-MS) For the Detection of Metal-Based Nanoparticles in Nanoelectronic Materials C. Stephan.

The use of nanoparticles in consumer products is showing a tremendous increase over time. The National Institute of Standards and Technologies reported that nanotechnology-based consumer products are currently entering the market at a rate of 3 to 4 per week, and it is estimated that $2.6 trillion in manufactured goods will contain nanotechnology in 2014[1]. In spite of their beneficial properties, possible risks for humans and the environment need to be thoroughly investigated, and multiple key characteristics must be assessed. Detailed information about the quantity, shape, size, size distribution, structure, composition, surface charge and functionality need to be studied and addressed in order to perform an appropriate and reliable risk assessment. This work presents Single Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS) as a tool in assessing the fate of engineered nanoparticles in environmental sample types, from tracking transformation/dissolution in surface water to bioaccumulation in L. variegatus, a species of aquatic worm [2-4]. The technique allows for the differentiation between ionic and particulate signals, measurement of particle sizes and size distribution, and assists in monitoring agglomeration. [1] National Institute of Standards and Technologies (NIST), Environmental Leaching of Nanoparticles from Consumer Products http://www.nist.gov/mml/ed/inorganic/leachnano.cfm (accessed October 11, 2013). [2] Assessing the Fate of Silver Nanoparticles in Surface Water using Single Particle ICP-MS http://www.perkinelmer.com/ [3] Quantitative Evaluation of Nanoparticle Dissolution Kinetics using Single Particle ICP-MS: A Case Study with Silver Nanoparticles http://www.perkinelmer.com/ [4] Analysis of Nanoparticles in Biological Tissues using SP-ICP-MS http://www.perkinelmer.com/

354 TRACKING NANOMATERIALS THROUGH THE LAUNDRY WASH CYCLE: RELEASE, DISSOLUTION AND COMPLEXATION D.M. Mitran, EMPA Technology & Society Lab / Environmental Risk and Management; B. Nowack, EMPA In the context of assessing the potential risks of nanomaterials, a lifecycle approach (covering production, use and disposal of the nanomaterial or nano-product) represents a holistic view of their potential impacts. Considering nanomaterial life cycles are determined by application within a product, relevant exposure scenarios and particle aging/transformations are strongly dependent on the life-cycle of nano-enhanced products themselves. For example, nanomaterials embedded in textiles tend to degrade or transform after exposure to oxidants and detergents during washing. Building on previous results which demonstrated the quantity and general size fractions of various silver additives released from textiles, we further characterized specific nanoparticle properties in the laundry wash cycle. Using a number of different liquid and powdered commercial detergents, washing studies were preformed with nanoparticles suspended in wash solution or released from laboratory prepared textiles with known nanoparticulate additives to better understand particle release, (change to) particle size distribution, dissolution, re-precipitation (i.e. “new” particle formation) and complexation of manufactured nanomaterials to particulate matter in the washing solutions, such as zeolite. Au NPs were used as a “tracer” through the system, since it is less reactive or prone to dissolution, to ensure analytical characterization techniques did not distort particle size or number concentrations throughout the tests. TEM and EDX analysis were performed to observe morphological and chemical changes to the particles after washing. Single particle ICP-MS (spICP-MS) was used to build a size distribution of particles in solution and detect complexation with larger floc material in the wash solution. When various conventional and nano materials were washed under standardized washing conditions, similar particle forms were detected regardless of the starting material. In contrast, varying the washing solution chemistry was found to dictate the extent and rate of dissolution, in addition to various surface chemistry changes detected depending on the concentration of bleaching agent. These studies help to further the understanding of nanomaterials through the product life cycle and give a better indication of how nanomaterials will be aged and transformed over time during regular nano-enhanced product use, not just in laboratory studies.

355 Environmental leaching of nanoparticles from a consumer product and their detection by single particle ICP-MS F. Von der Kammer, Vienna University / Department of Environmental Geosciences; J. Navratilova, A. Gondikas, University of Vienna / Environmental Geosciences; T. Hofmann, University of Vienna / Department of Environmental Geosciences.

The increased use of products containing nanoparticles will inevitably lead to their release into the environment. Currently, the most commonly used nanoparticles in consumer products are metal oxide nanoparticles. Single particle ICPMS (spICPMS) has already showed a great potential for tracing silver nanoparticles in the aquatic environment, and it is the most promising technique for routine analysis of metallic nanoparticles in environmentally relevant concentration levels (ng/L). The biggest challenge in spICPMS analysis is to achieve the lowest particle size detection limit which is directly related to the detection limit, what puts high demands on the ICPMS instrumentation. In our study the triple quadrupole ICP-MS in single particle mode is employed to track potential release of iron oxide nanoparticles from a consumer product into the simulated aquatic environment.

356 Detection of engineered cerium oxide nanoparticles in soil extracts A. Praetorius, University of Vienna / Department of Environmental Geosciences; S. Wagner, University of Vienna / Department of Environmental Geoscience; W. Fabienke, M. Velimirovic, University of Vienna / Department of Environmental Geosciences; E. Neubauer, University of Vienna / Environmental Geosciences; R. Kaege; F. Von der Kammer, Vienna University / Department of Environmental Geosciences; T. Hofmann, University of Vienna / Department of Environmental Geosciences.

Engineered cerium oxide nanoparticles (CeO2 NPs) are expected to be released to the environment during their application as e.g. diesel fuel additives or automotive catalytic converters. There is a clear need to quantify CeO2 NPs in environmental matrices in order to assess their potential risks, but the detection of engineered CeO2 NPs in complex natural media is very challenging due to the low expected CeO2 NP concentrations and the comparatively high background of Ce-containing minerals of similar size range. We here present a new analytical method, based on single particle (sp) ICP-MS analysis for identification and quantification of engineered CeO2 NPs. We expect pulse signals of natural Ce-containing particles to be low and to not represent the true size of the particle detected by the sp-ICP-MS. In contrast, engineered CeO2 NPs will appear as a spike which is significantly higher than the background signal and can be used to determine the mass and number concentration as well as the particle size of the CeO2 NPs. Our hypothesis was tested with a set of experiments under increasingly more complex (and realistic) conditions. More specifically the following combinations of engineered CeO2 NPs (Sigma Aldrich, CoO)
fullerenes. The flow rate was kept low (0.1 mL/min) using a slot pump. To separate
the fullerenes in the FFF, several eluents were tested. A solution of
0.05%(v/v)% NH$_2$OH was identified as a good eluent for the separation of three
different fullerenes (C$_{60}$, C$_{60}$-bisphenyl butyric acid methyl ester (60PCBM)
and C$_{60}$-bis(bisphenyl)-butyric acid methyl ester (bisbiscPM)). The size
distribution received with this eluent coincides with literature values, the FFF
theory and a calibration curve. This development of a diagnostic data fullerene
allowed to analyse different water types spiked with fullerenes. Different size distributions for
fullerenes in MilliQ drinking water, influent and effluent were found. It is obvious that
the water matrix does effect the size of the clusters significantly.

358 Investigating nanoparticle transformation in situ in the environment through the use of nIDDs
E. Lombi, University of South Australia / Centre for Environmental Risk
Assessment and Remediation; M. Khaksar, University of South Australia; R.
Sekine, E. Donner, University of South Australia / Centre for Environmental Risk
Assessment and Remediation; D.F. Jolley, University of Wollongong; K. Vasištek,
University of South Australia / Masnow Institute; S. Kirk, US EPA.
Over the past two decades, extremely rapid progress in nanomaterial research and development has
been met by equally rapid commercialisation of new products and technologies. As a result, manufactured nanoparticles (sub-100 nm scale) are
increasingly released, both directly and indirectly, to the environment.
Unfortunately, our understanding of the fate of manufactured nanomaterials in the
environment is extremely deficient. In particular, the transformation of
nanoparticles under natural conditions is a very challenging area of research due to the
environmental low concentrations that can be expected at present time. In this study we report on the development and testing of a device, nano in situ deployment device
(nIDD), in which plasma polymerization is used to immobilize Ag-NPs on a
substrate which allows us to analyse nanoparticles upon exposure and retrieval
in a different complex environmental compartments. Plasma polymerization was
used to immobilize the Ag-NPs on a solid substrate. Plasma polymerization is a
technique which allows surfaces to be modified with a thin functional polymer
layer. These functional films can be applied on any type of substrate material. In
this study, nanoparticles were immobilised on plasma polymerised Kapton tape
supported by a polycarbonate frame with an internal window, of variable size
(1-12 cm$^2$). The nIDDs were exposed to a range of environmental conditions
including a freshwater lake and in a marina, freshwater and saltwater sediments, a
sewer system in Adelaide and to the atmosphere in a number of cities in Australia,
Asia, Europe and the US. Upon retrieval, the chemical speciation of Ag
on the exposed devices was determined by X-ray Absorption Near Edge Structure
(XANES). In technical research, environmental compartments are different and physical conditions play a dominant role in determining Ag speciation. In most
cases, complexation of Ag by reduced sulfur groups is the key transformation
mechanism but variability exists within various compartments. This study demonstrates that nIDDs can be also used to explore NP transformation in highly
dynamic and spatially heterogeneous environments.

Adverse outcome pathway concept in research and risk assessment

359 Quantitative AOP Linking Aromatase Inhibition to Impaired Reproduction: A Case Study in Predictive Ecotoxicology

The adverse outcome pathway (AOP) framework is intended to help support greater use of mechanical toxicology data as a basis for risk assessment and/or regulatory decision-making. While there have been clear advances in the ability to rapidly generate mechanistically-oriented data for large libraries of chemicals using in vitro high throughput assays, questions remain about whether the AOP framework can be used to help translate those data into quantitative predictions of the probability or severity of an adverse outcome. This study couples together a series of computational models to yield a quantitative AOP (Q-AOP) construct capable of
predicting plasma vitellogenin concentrations yielded markedly different
dose-response time-course behaviors and subsequent population trajectories. These results suggest that model uncertainty could be reduced considerably through additional iterations of model testing and refinement. Nonetheless, the case study supports the feasibility of using computational representations of generalized biological response motifs in the form of a Q-AOP as a basis for translating
in silico results generated via high throughput screening into meaningful
predictions of ecotoxicologically-relevant outcomes. The contents of this presentation neither constitute nor necessarily reflect US EPA policy.

360 Cheminformatics approaches for defining Molecular Initiating Events within and Adverse Outcome Pathway for Chronic Toxicity
S.J. Enoch; M.D. Nelms, F.P. Steinmetz, C.L. Mellor, A. Richarz, Liverpool John Moores University / Centre for Environmental Risk Assessment; J. Madden, Celcis Invitro Technologies; M. Cronin, Liverpool John Moores University.
The Adverse Outcome Pathway (AOP) approach centres on identifying and understanding the key, testable, steps in a biological pathway that when perturbed leads to a toxicological response. It is envisaged that the identification of key events will allow for the development of intelligent testing strategies consisting of in silico, in vitro and in chemico methods. One of the most challenging endpoints for the AOP paradigm is chronic repeated dose toxicity, following exposure of humans or environmental species to a toxicant. Within the AOP approach, cheminformatics methods enable knowledge concerning the Molecular Initiating Event (MIE) to be extracted from large chemical databases. These properties of the chemical are then prioritised for further, non-animal, testing or grouped into categories
allowing data-sets to be filled via read across; this offers a potential route for
non-animal safety assessment in a number of industries. This presentation will outline the development of two profilers for organ-toxicity where the MIE is either
1) the transport either, protons across the mitochondrial membrane, or electrons
directly from complex I to complex IV in the respiratory chain or 2) the inhibition
of the nuclear receptor family of proteins. Where MIEs are conserved across species this also presents an opportunity to predict toxicological effects in species for which empirical data are unlikely to be available. This work describes the development of in silico profilers, based on knowledge of the MIE that can be used to predict organ-level toxicity. Whilst the focus of this study relates to human toxicity, the potential for extrapolation across species, using knowledge of the MIE, is also discussed.

361 Development of an adverse outcome pathway from bile salt export pump inhibition to cholestatic injury
M. Vinken, Vrije universiteit Brussel / Toxicology; B. Landesmann, European Commission DG Joint Research Centre; S. Vinken, Algemeen Stedelijk Ziekenhuis Aalst / Department of Endocrinology; H. Jaeckch, University of Kansas Medical Center / Department of Pharmacology Toxicology and Therapeutics; C. Willett, the Humane Society of the United States / Animal Research Issues; M. Whelan, European Commission DG Joint Research Centre / Department of Pharmacology Toxicology and Therapeutics; D.F. Jolley, University of Wollongong; K. Vasilev, Cranfield University; M. Hecker, University of Saskatchewan / School of the Environment and Sustainability and Toxicology Centre; K. Tollefsen, NIVA / Norwegian Centre for Environmental Assessment and Remediation; M. Khaksar, University of South Australia / Mawson Institute; S. Kirk, US EPA; M. Vinken, Vrije universiteit Brussel / In Vitro Toxicology and Dermatocosmetology
Adverse outcome pathways (AOPs) have been recently introduced in human risk
assessment as pragmatic tools with multiple applications. As such, AOPs intend to
provide a clear-cut mechanistic representation of pertinent toxicological effects.
AOPs are typically composed of a molecular initiating event (MIE), an intermediate step and key events, and an adverse outcome. In this study, an AOP framework is proposed for cholestasis triggered by drug-mediated inhibition of the bile salt export pump transporter protein. For this purpose, an in-depth survey of
relevant scientific literature was carried out in order to identify intermediate steps and key events. The latter include bile accumulation, the induction of oxidative stress and inflammation, and the activation of specific nuclear receptors. Collectively, these mechanisms drive both a deteriorative cellular response, which underlies directly caused cholestatic injury, and an adaptive cellular response, which is aimed at counteracting cholestatic insult. AOP development was performed according to Organisation for Economic Co-operation and Development (OECD) guidance, including careful consideration of the Hill criteria for the selection of evidence assessment endpoints and the OECD key questions for evaluating AOP confidence. The postulated AOP is expected to serve as the basis for the development of new in vitro tests and the characterization of novel biomarkers of drug-induced cholestasis.

362 Potential Roles of Omics Data in the use of Adverse Outcome Pathways for Environmental Risk Assessment
G. Hodges, Unilever Research / Safety and Environmental Assurance Centre; F. Falciani, University of Liverpool / Functional Genomics; G.T. Ankley, U.S. EPA / National Health and Environmental Effects Research Laboratory; E. Butler, Cranfield University; M. Hecker, University of Saskatchewan / School of the Environment and Sustainability and Toxicology Centre; K. Tollefsen, NIVA / Ecotoxicology and Risk Assessment; N. Garcia Reyero, Mississippi State University; P. Kille, Cardiff University; D. Becker, University of Birmingham; E.K. Brockmeyer, University of Liverpool / Functional and Comparative Genomics;
in the process of determining the distribution of narcotic compounds across different membrane types (cell membrane versus mitochondrial membrane), and of evaluating *in vitro* tools for the assessment of narcotic effects.

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**Cause and effect: drug plasma concentrations drive the multi-level effects of glucocorticoids**


Synthetic glucocorticoids (GCs) have important clinical utility both as anti-inflammatory and immunosuppressive agents. These compounds act by modulating the glucocorticoid receptors (GR), but can also interact with other non-classical GR targets (off-targets), including the androgen receptors (AR). Recent studies reported GR activity in wastewater effluents and surface waters, but this activity could not be explained by the chemical analysis of known glucocorticoids. Considering the key role of GR in physiological processes, understanding the consequences of the disruption of GR signalling in non-target species is a priority. Despite the likely source, GR activity in the environment has not been linked to any known pharmaceutical. In this context, the characterization of the pharmacological and toxicological effects of model GCs in fish could provide an important knowledge-platform to predict the potential effects of GR activity detected in the environment. Here we provide an integrated assessment of the multi-scale effects of beclomethasone dipropionate (BDP) in the teleost fathead minnow (*Pimephales promelas*) anchored to drug plasma concentrations. The endpoint selection was driven by the Read-Across toolbox and by the cross-species extrapolation of clinical and pre-clinical data. The expression of a set of five genes was linked to relevant endpoints at higher level of biological organization. In particular, the gene expression of GR, AR, phosphoenolpyruvate carboxykinase (PEPCK), interleukin-6 (IL6) and tumor necrosis factor alpha (TNF-α) was linked to the expression of secondary endpoints (characteristics, levels of plasma glucose, and effects on white blood cells population. The latter set of endpoints corresponds to typical adverse or therapeutic effects occurring in humans: e.g. skin androgenisation, hyperglycemia, and immunosuppression. The effects were characterized by performing two independent 21-day exposure experiments in flow-through conditions during which adult fish were exposed respectively to 10, 100 and 1000 ng/L (Experiment 1) and 10 and 1000 ng/L (Experiment 2). The two experiments were designed in order to achieve the same peak blood concentration (*C_{max}*) but different drug-concentration-time profiles (Area Under the Curve, AUCs), which reflect the actual body exposure to the drug. We will discuss the results of these studies and the implications for both Environmental Risk Assessment of GCs and Adverse Outcome Pathway concept.

Midpoint or single score for decision making? (II)

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**A Normalization Approach for Simplified Ecodesign Tools in the Food Sector**

U. Schenker, Nestle Research Center; N. Espinoza-Orias, Nestec Ltd. / School of Chemical Engineering and Analytical Science

Nestlé, the world’s leading Nutrition, Health, and Wellness company has, in the past six years, used simplified ecodesign tools to evaluate the environmental impacts of over 15,000 packaging design alternatives. Today, a new eco-design tool called EcodEX has been developed and implemented which extends the assessment process to also evaluate food products. Users of the tools are typically packaging or food scientists with limited background in LCA and environmental sciences. All assessments cover between five and eight environmental impact indicators, typically at midpoint level. Decision making is a challenge for the tool users, because it is difficult for them to judge which indicators “matter most” for any given project. Several approaches have been tested to overcome this: using “intuitively” understandable equivalency units for LCA indicators (such as “number of showers” for liters of water, or “km driven by car” for kg CO₂eq), and providing qualitative rules of thumb (such as: GHG emissions matter for products with diary ingredients, water use matters for products with use of irrigation in agriculture). Neither approach was fully satisfactory. A new normalization approach has been recently implemented which is inspired by guidance on nutrition Recommended Daily Intakes (RDI). The approach normalizes the existing environmental indicators with the average daily impacts of one person in Europe. The resulting number, which is a percentage, is easy understandable by packaging & food scientists, as it is closely related to the Recommended Daily Intake in nutrition. This approach easily allows the identification of those indicators that matter most for any given product system. For instance GHG emissions were shown to play an important role in dairy products (related to enteric fermentation in cattle), as was water consumption for coffee-related products (given that irrigation is frequently used in green coffee cultivation). While the different normalized indicator scores are not summed together, the fact that they are displayed next to
each other implies that equal weighting factors are used for each of the indicators, without aggregating to a single score. The proposed approach is much more transparent than single score indicators because it can still easily be seen which design alternative performs better with regards to the different indicators.

366 The PROSUITE (aggregation) methodology for sustainability assessment: lessons learnt from the biorefinery case study  
S. De Meester, Ghent University / Department of Sustainable Organic Chemistry and Technology; M.K. Patel, University of Geneva / UNIGE; J. Dewulf, EC JRC - IES / Sustainability Assessment unit

In the FP7 project PROSUITE, Prospective Sustainability Assessment of Technologies was developed that quantifies impact on the three dimensions of sustainability: People, Planet, Profit including both micro and macro scale effects. The final PROSUITE framework consists of a selection of 40 midpoint indicators of relevant social, economic and environmental aspects and groups this into 5 endpoints, namely: impact on social well being, prosperity, exhaustive resources, natural environment and human health. Background data has very different origins: e.g. ecoinvent, input output tables, ILO statistics, etc. Aggregation, required to reduce complexity and allow decision making is thus a big challenge in such an assessment method. Ideally, the different midpoints could be linked by a scientifically sound cause-effect chain, however, whereas this endpoint modelling is already relatively uncertain in quantification of impact on natural environment this becomes much more complex when trying to include other dimensions and aspects. The endpoint weighing is a compatible method. The calculated midpoint weighting factors based on endpoint methods. All other factors are radically different. While midpoint weighing is not the first choice for the used data on endpoints units, the weighting between midpoints and endpoints and subsequently the aggregation to a single sustainability index. Finally conclusions are formulated on how such a complex assessment methodology should be interpreted and communicated.

367 What if we would use endpoint results for estimating midpoint weighting factors?  
T. Ponsioen, PRe Consultants / Consultancy; M. Goedkoop, PRe Consultants

There is often a preference for communicating life cycle assessment results at midpoint level, because endpoint methods are generally considered as highly uncertain. However, the importance of the midpoint on the different midpoint categories is difficult to determine without considering the information in endpoint methods. While the uncertainty of endpoint results is high, their scientific approach could provide a more solid basis for midpoint weighting factors than value judgment. This paper shows how to estimate midpoint weighting factors based on the endpoint single score results of the same impact assessment method framework and present the results. The ReCiPe method was chosen because it contains a large set of midpoint and endpoint categories. Water scarcity categories were added from a compatible method. The calculated midpoint weighting factors based on endpoint scores show a similar importance of climate change compared to the stakeholder weighting. All other factors are radically different. While midpoint weighing schemes based on panels usually have a range of factors lower than a factor 15, we notice a difference on the same order of magnitude. There are different midpoint factors so uncertain that they must be wrong or the way a panel assesses midpoint weighting is flawed. The problem in midpoint panel procedures is well understood in social science. There are a number of issues that are often overlooked when panels are used: a) there are too many issues at midpoint, b) the way the questionnaire is formatted creates biases, c) people have different experiences (observed, perceived or predicted), and d) people relate to what they understand (environmental issues are difficult to understand). It must be noted that stakeholder weighting is used at endpoint level, but this involves fewer issues and sensitivity analysis shows the results are robust. Using midpoints as environmental impact indicators are useful for benchmarking and monitoring. However, determining the relevant impact categories is challenging when working with midpoints. When stakeholders are asked how to assign weighting factors on midpoint category, they will deviate extremely from those obtained with endpoint methods. Stakeholder weighting at midpoint is per definition unreliable and should be avoided. Weighting factors based on endpoints will provide decision makers with much more realistic information on which categories are important for the studied product.

368 LCA for decision support  
F. Dinkel, T. Kagí, Carbotech AG

Weighting the different LCIA indicators to form a single score indicator is a particularly necessary step for different purposes e.g. to evaluate the relevant impact categories considered in a LCA or in a comparison of different options, in order to base a decision not only on one or few indicators but to take all different indicators into account. This is required when the various indicators do not lead to the same conclusions. On the other hand it is well known that weighting is based on value choices and not on natural science. That is why according to ISO weighting shall not be used for comparative assertions disclosed to the public. So what should be done if a recommendation has to be given to customers e.g. from authorities? What will be the consequences if decisions are strictly based on ISO? The big advantage of the different midpoints is that the LCA practitioner gets an insight into the different impacts and can also take the local situation into account for the conclusions. So he has to substantiate the conclusions. This is why, the relevance of the different indicators is crucial and single score methods based on consensus and not only on personal opinions are not only helpful but necessary. As the analysis of the weighting methods of the last thirty years has shown, the weighting corresponds to the actual knowledge, political topicality and social awareness. In order to reach a sound decision, different single score methods should be used to conduct sensitivity analysis, which allows assessing the consequences on the LCIA results due to different value-choices of the weighting methods. Single score indicators do not tell the truth but can be very helpful when it comes to decision support and to communicate an outcome. However it is crucial not to use only one single method for the conclusions and that the values behind as well as the coverage is known and adequate to the goal and scope of the study. So we suggest completing the requirement in the norm as follows "More than one single score method shall be used for comparative assertions disclosed to the public and the weighting has to be discussed."

369 Panel discussion: using midpoint or single score indicators in LCA  
T. Kagí, Carbotech AG

370 Panel discussion (Ctd.): using midpoint or single score indicators in LCA  
T. Kagí, Carbotech AG

Risk assessment of chemical mixtures: trends, developments and bottlenecks for integrating exposure and effects

371 Mixture toxicity assessment of biocides - from theory to practice: state of play and analyses for 30 products  
A. Kehrein, Federal Environment Agency / IV Biocides; A. Coors, ECT Oekotoxikologie GmbH; F. Sacher, DVGW-Technologiezentrum Wasser; B. Schwarz-Schulz, Federal Environment Agency (Umweltbundesamt, UBA)

Biocidal products are typically mixtures of one or more active substances as well as further ingredients. For the authorisation of biocidal products an extensive environmental risk assessment (ERA) is required in accordance with the biocidal products regulation (BPR, 528/2012), i.e. not only every active substance in the product has to be subject of an environmental risk assessment, but also substances of concern, e.g., substances leading to classification of the product or having PBT, endocrine or CMR properties, have to be evaluated separately. In addition the combined toxicity of these ingredients needs to be taken into account according to the legal text. From 2011 until 2013 a proposal for the (environmental) risk assessment of the mixture toxicity of biocidal products was developed under the lead of Germany which was agreed between member states and industry at technical level and is already published at ECHA’s website as transitional guidance. The starting point was the so-called "pattern of concern", which extends from theory to practice: state of play and analyses for 30 products. A results overview of a results database was presented in context of the transitional guidance document for the mixture toxicity assessment of biocidal products especially regarding the definition of relevant substances, i.e. product components that should be included in a mixture assessment.

372 Estimating the environmental risk of the societal stock of additives in plastics by a chemical footprint approach  
T.V. Rudberg, J. Lexén, IVL Swedish Environmental Research Institute; L. Dahlgren; P.A. Cousins, M. Rahmberg, IVL Swedish Environmental Research Institute; H. Anderson, JVL Swedish Environmental Research Institute Ltd / Department of Chemical and Biological Engineering; J.E. Grönholdt Palm, Department of Studies in Environmental Science; U. Sahlin, Linnaeus University, School of Natural Sciences
Environmental risk posed by additives in products in the technosphere in general and in consumer products in particular, is an important issue that has been, so far, investigated to a relatively limited extent. Previous estimates of the national stock and emissions are available on national scale [1,2,3] but the challenge has remained to understand how important or significant these stocks and emissions are from a risk perspective. The research presented here approaches the challenge by assigning risk characteristics to additives and evaluating the outcome with corresponding risk characterisation for biocides. We used the previous estimates on stock and emissions of plastic additives. Data for use of biocides were extracted from the Swedish Chemicals Agency. The risk characterisation was carried out by applying USETox characterisation factors (CF). The amount of each substance, has been multiplied with its corresponding CF from USETox. These substance specific risk parameters are then added to the overall risk score. It is clear that there are many uncertainties in the calculations. For example the emission calculations in the overall society example seem unreasonably high, in the order of 2% annually of the total stock. In other work applying an advanced emission model to a limited sample of products, the emission rate is in the order of 0.02% annually. This indicates that the overall society-wide emissions could be in the order of 500 tons rather than 47000 tons. For the risk scores, the uncertainty is even bigger, as the uncertainty of the CFs themselves come into play, as well as the incomplete availability of CFs. The total risk score for the National total use of biocides is 9.3E+09 CTU. This is to be compared with the risk scores as calculated for the additives, which is 1.3E+12 CTU for the total stock of additives and 3E+10 CTU for the emissions, or possible a factor 100 lower if assuming a similar overestimation as for emissions. The results are various with exclusively only one concentration and also lack CFs for possibly important substances, both among the additives and the biocides.

## 373 Environmental risk assessment of chemical mixtures: is a specific mixture assessment factor (MAF) the right way forward to improve current regulatory approaches?

T. Backhaus, University of Gothenburg | Department of Biological and Environmental Sciences; M. Gustavsson, Department of Biological and Environmental Sciences; A. Hartmanns, University of Gothenburg | Department of Biological and Environmental Sciences; M. Faust, Faust & Backhaus Environmental Consulting; M. Klun, H. Sundberg, S. Gabring, G. Ericson, S. Flodström, Swedish Chemicals Agency

Current regulatory frameworks risk-assess chemicals one at a time. It has been repeatedly pointed out that this approach is not sufficiently protective, as humans as well as environmental organisms are de facto exposed to complex chemical mixtures. It has been previously discussed to account for the increased toxicity of such mixtures of the risk assessment methods through additional Mixture Assessment Factor (MAF) of “n”, the number of components in the mixture. It can be proven that such a MAF is sufficiently protective under the assumptions that (i) the mixture behaves according to concentration addition, and (ii) that none of the components is present at a concentration that poses an individual risk. However, such a MAF might become overly conservative in situations where only a few components dominate the expected toxicity of a mixture and it (obviously) requires to provide an estimate of the actual number of components in the mixture. These assumptions and limitations pose the question whether and in which situations a default MAF of “n” might be a suitable approach to account for mixture toxicities in regulatory risk assessment, or whether there are alternative approaches available – which will be discussed in the presentation. In order to identify the appropriate size of a MAF for environmentally realistic mixtures, we selected specific mixture scenarios from the open literature and used them as basis for simulation studies. The impact of specific toxic unit distributions, modes and mechanisms of action of the mixture components, (eco)toxicological endpoints and exposure scenarios will be discussed.

## 374 Mixture Toxicity Prediction via a Quantitative Toxicogenomic-based Approach

N. Gou, Northeastern University | Civil and Environmental Engineering; A. Guo, Department of Civil & Env Engineering | Department of Civil | Env Engineering Biotechnology initiative Research

The mixture toxicity has been a major concern and longstanding research topic in water toxicity assessment. Recent years, toxicogenomic approaches provide tools to improve our understanding and predictability of the mixture toxicity. However, current evidence to support the published mixture assessments from toxicogenomic studies is as yet mainly observational and needs further improvement. Few existing situations can simulate the interaction of the mixture impacts, and the combined effects is lacking. In this study, we demonstrated the application of a toxicogenomic-based approach for investigating the combined effects of various binary mixtures with a range from environmental relevant concentration to the benchmark level. Five binary mixtures were investigated at various concentrations with a fixed ratio (w/w). Cellular level responses (indicated by our quantitative omic-index TELLI) evoked by most of the binary mixtures exhibited clear dose-response patterns as single chemicals did. The TELLI values were studied using the 4-parameter logistic regression model for further analysis. To quantitatively analyze the combined effects of mixtures, two additive models were applied – concentration addition (CA) and independent action (IA). Parallel dose-response curves were found among As, Cr and the mixture, which indicate similar mode of action (MOA), and the experimental observed mixture toxicity fit well to both CA and IA model prediction (r²>0.8). For mixture of NDMA - atrazine, mitomycin – erythromycin, experimental observed toxicity fit better with IA model which may indicate dissimilar MOA. Significantly higher experimental toxicity than both model predications was found the mixture of H2O – saccharose indicating possible synergistic effect. And significantly lower experimental toxicity than both model predications was found for mixture of As – Cr – saccharose indicating partial antagonistic effect. Furthermore, for the last two mixtures, the combined effects seem to be dose-dependent. Our results not only illustrated a rapid, sensitive, and informative approach for mixture toxicity studies, but also for the first time demonstrated the possibility to explicitly predict the combined effects at the molecular level (when compounds are present at high concentration). Although most of the mixture exhibited additive effect in our results, further investigation is required for better understanding of the factors that influence the combined effects.

## 375 Azoles from Different Regulatory Frameworks: Combined Assessment with Regard to Wastewater Effluents


While the environmental risk assessment of human pharmaceuticals, biocides, plant protection products, cosmetics, and industrial chemicals is regulated in different legal frameworks in the European Union, these substances may be released together to surface waters via wastewater treatment plants. Hence, the consideration of the potential combined toxicity of these substances and the resulting environmental risks may need to be evaluated across different regulatory frameworks. Azoles were selected as suitable example for a combined environmental risk assessment across regulatory boundaries based on the concept of concentration addition as they share the fungicidal mode-of-action and are used as pharmaceuticals, as plant protection products, as biocides, and in products regulated under REACH. The present study investigated the individual and combined effects of three azoles in the absence and presence of diluted wastewater effluent to evaluate if conclusions drawn from testing artificial mixtures in the laboratory can be transferred to real-world mixtures. The observed toxicity of equipotent mixtures prepared in test medium deviated from the predicted toxicity by less than factor 5. Although there was a trend rather of overestimation of combined toxicity than underestimation, these results generally support the applicability of the concept of concentration addition for risk characterization scores to the additives and comparing the outcome with risk perspective. The research presented here approaches the challenge by assigning regulatory protection products, cosmetics, and industrial chemicals is regulated in different legal frameworks in the European Union, these substances may be released together to surface waters via wastewater treatment plants. Hence, the consideration of the potential combined toxicity of these substances and the resulting environmental risks may need to be evaluated across different regulatory frameworks. Azoles were selected as suitable example for a combined environmental risk assessment across regulatory boundaries based on the concept of concentration addition as they share the fungicidal mode-of-action and are used as pharmaceuticals, as plant protection products, as biocides, and in products regulated under REACH. The present study investigated the individual and combined effects of three azoles in the absence and presence of diluted wastewater effluent to evaluate if conclusions drawn from testing artificial mixtures in the laboratory can be transferred to real-world mixtures. The observed toxicity of equipotent mixtures prepared in test medium deviated from the predicted toxicity by less than factor 5. Although there was a trend rather of overestimation of combined toxicity than underestimation, these results generally support the applicability of the concept of concentration addition for risk characterization scores to the additives and comparing the outcome with risk perspective. The research presented here approaches the challenge by assigning regulatory

## Soil and water pollutants’ assessment, monitoring and remediation (III)

### 376 Transfer of emerging pollutants from Spanish biosolids used as agricultural soil amendments. Results of CECAREA project

A. de la Torre, Environmental; I. Navarro, P. Sanz, CIEMAT; M. Porcel, F. Pro, G. Carbonell INIA; M. Martínez, CIEMAT

The use of the organic fraction of sewage sludge and municipal solid waste compost (MSW) as agricultural amendment, offers benefits and acceptable risks to both soil and plants is one of the best outings environmentally sustainable waste. Nevertheless, it is important to emphasize that contaminants like heavy metals, pathogenic microorganisms and much of the organic pollution ends up in wastewater and in the MSW compost. The pathogens can be removed by various treatments, but there are some organic compounds that do not break down easily during wastewater treatment and / or MSW composting, and tend to accumulate in biosolids. Consequently, if they are used for agricultural purposes, could originate problems due to toxicity, bioaccumulation and transfer through the food chain. Responding to this concern, scientific community has made important efforts to characterize waste, by detecting some emerging pollutants (halogenated flame retardants and perfluorinated chemicals) in this kind of biosolids. Nonetheless, there is still a lack of knowledge regarding their fate, transfer or potential bioaccumulation after soil amendment. For this reason, Spanish Ministry of Economy and Competitiveness funded the “CECAREA” project to evaluate environmental consequences of utilizing biosolids as agricultural soil amendments. In order to identify the appropriate size of a MAF for environmentally realistic mixtures, we selected specific mixture scenarios from the open literature and used them as basis for simulation studies. The impact of specific toxic unit distributions, modes and mechanisms of action of the mixture components, (eco)toxicological endpoints and exposure scenarios will be discussed.


377 The sorption mechanism of sulfonamide antibiotics on humin and its subfractions extracted from peat soil
X. Guo, Peking University / College of urban and environmental science; X. Wang, Peking University; S. Tao, Peking University / College of Environmental Sciences; B. XING, Department of Plant, Soil and Insect Sciences / University of Massachusetts

Chromolaena odorata (L) King and Robinson is known as the strongly associated organo-mineral composite formed by natural organic matter in soils. Until now, the role of humic acid and fulvic acid as sorbents for organic contaminants has been extensively studied. In contrast, the research concerning on the sorptive of the HM fraction is far from enough, even though it typically represents more than 50% of the organic carbon in soil. The sulfonamide antibiotics given to livestock are poorly metabolized and absorbed, thus a large fraction is released into the soil environment, causing the proliferation of antimicrobial-resistant pathogens and further affecting humans. Their sorption to soil organic matters (SOMs) is a critical process controlling their transport, bioavailability, and reactivity in soil. Therefore, to probe sorption mechanisms between sulfonamide antibiotics and humin components is of great importance. In this study, two kinds of high-use sulfonamide antimicrobial, sulfamethazine (SMT) and sulfamethoxazole (SMX) were chosen as subject compounds. Four procedures were used to remove ash, carbohydrate, lipid and lignin component from original humin sample to investigate the impact of each component on sorption more clearly and confidently. After e-ashing treatment, both surface and bulk polarity of humin samples decreased, leading to the increase of their sorption for both SMT and SMX. The experimental apparatus, i.e., the newly Microbial Fuel Cell (mMFC), one of these procedures, thus the more hydrophobic organic groups that were previous masked by minerals become available to sorbates. Those hydrophobic organic moieties could interact with sorbates via van der Waals force, and also form pi-pi bond with the benzene ring in the molecule of SMT and SMX, thus enhancing the sorption intensity. Similarly, the removal of lipid and lignin components enhanced the sorption for both SMT and SMX as well. However, the sorption increment was different from the de-ashing process. These organic components may block high affinity sorption sites in the interior part of the humin. The condensed organo-mineral complex components inside HM became available to the sorbates after the removal of lipid and lignin components. The sorption nonlinearity for SMT and SMX also enhanced after each treatment. Those components in humin removed by chemical procedure were comparatively homogeneous, while leaving those highly heterogeneous components in the residue material, resulting in high sorption nonlinearity.

378 Supporting microbial activities by using electrodes as electron acceptors: strategies to biomimiclarize 14C herbicides in soils
J. Rodrigo, Helmholtz centre for environmental research - UFZ / Soil Department; A. Domínguez, Universidad de Alcalá; U. Dörfler, R. Schroll, Helmholtz Zentrum München / Institute of Soil Ecology; A. Estévez-Núñez, University of Alcalá

Biocatalytic systems (BES) show tremendous potential for utilization in environmental applications. So the newly Microbial Fuel Cell (mMFC), one of these BES, use electrodes to supply the electron acceptors required for contaminant biodegradation in contaminated soils, where the organic pollutants play the fuel role for microbial electrochemical communities. We propose to name this kind of bioelectrochemical devices as Microbial Remediation Cell (MRC/unPolycyclic aromatic hydrocarbons as dibenzothiophene (DBT), and chloroaromatic compounds as the herbicides diuron and atrazine were shown to be efficiently biodegraded under electrolysis conditions in our laboratory, showing rates 10-times faster by using electrogenic approaches in contrast with the natural biodegradation. Here, we will review the results of our recent investigation on the use of MFCs for in situ bioremediation of 14C herbicides. Radio-labelled compounds allow us to control the pollutant fate and establish a strict mass balance of 14C. For monitoring these processes, we have design a special set up, adapting the MRC to a closed laboratory system, being possible to maintain total control of the overall behavior of the pollutant and to use on chronovoltammetry and chronoamperometry for measuring electrons released from the pollutant microbial oxidation to the anode. The effective bioremediation task was confirmed by measuring the 14C radioactivity in the electron acceptors extraction fractions and to identify 14C-metabolites. At last, the residual radioactivity bound to the soil is determined by combustion processes, leading defined all terms for the mass balance of 14C. The results support the concept of electrogenesis-stimulated bioremediation, being intensely increased the mineralization of 14C IPU under electrolysis conditions (MERCs with anode at 0.6 V vs NHE) with the parallel change in the metabolic pathway. Consequently, the intrinsic soil capability to mineralize IPU under flooded conditions was enhanced by ca. 40-fold, reaching high mineralization rates even at the initial stage of the experiments.

379 Phytotreatment of polychlorinated biphenyls contaminated soil by Chromolaena odorata (L). King and Robinson
R.O. ANYASL, University of South Africa / Environmental Sciences; H.I. Atagana, University of South Africa / GRADUATE STUDIES

In this study, phytotreatment ability of a weed on Aroclor 1254 was studied under greenhouse conditions. Chromolaena odorata plants were transplanted into soil containing 100, 200, and 500 ppm of Aroclor in 1L pots. The experiments were watered daily at 70 % moisture field capacity. Parameters such as fully expanded leaves per plant, shoot length, leaf chlorophyll content as well as root length at harvest were measured. PCBS was not phytotoxic to C. odorata growth but plants in the 500 ppm treatment only showed diminished growth at the sixth week. Percentage increases in height of plants were 45.9, 39.4 and 40.0 for 100, 200 and 500 ppm PCBS treatments respectively. The number of nodes, root number, length and leaf chlorophyll concentration. The control sample showed 48.3 % increase in plant height which was not significant from the treated samples, an indication that C. odorata could survive such PCB concentration and could be used to remediate contaminated soil. Mean total PCB absorbed by C. odorata plant was between 6.40 and 64.60 ppm per kilogram of soil, leading to percent PCB absorption in the range of 0.006 - 0.013 % per kilogram of contaminated soil. PCBS were found mostly in the root tissues of the plants, and the Bioaccumulation factor were between 0.006-0.38. Total PCB absorbed by the plant increases as the concentration of the compound is increased. With these high BAF ensured, C. odorata could serve as a promising candidate plant in phytoextraction of PCB from a PCB-contaminated soil. Keyword: Phytoextraction, Bioremediation, Soil restoration, Polychlorinated biphenyls (PCB), Biological treatment, Aroclor.

380 Climate change effects on PAH photodegradation in Mediterranean soils
M. Marques, Rovira i Virgil University / Chemical Engineering; M. Mari, Rovira i Virgil University / Chemical Engineering; J. Sierra, Universitat Rovira i Virgili / Chemical Engineering; J.L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; M. Nadal, University Rovira i Virgili / Laboratory of Toxicology and Environmental Health

The potential of climate change to alter the environmental distribution and biological effects of chemical toxicants, such as persistent organic pollutants (POPs) and other semi-volatile organic compounds, have been mentioned during the last years. Polycyclic aromatic hydrocarbons (PAHs) are a family of widespread environmental pollutants, whose chemical structure is based on two or more fused benzene rings. PAHs are released from natural and anthropogenic combustion processes. Environmental fate and transport of PAHs is highly influenced by temperature and solar radiation. Thus, PAHs are likely to be impacted by changes in temperature and radiation related to climate change. This study was aimed at estimating the loss rate of PAHs by means of photodegradation in two typical Mediterranean soils, after comparing two climate scenarios. Soil samples were taken from the A horizon of two common Mediterranean soils located in remote areas. Arenosol soil samples corresponded to Haplic Arenosol, an acidic and coarse-textured soil with granitic origin. In turn, Regosol soil samples belonged to Calcaric Regosol, which is formed of sedimentary materials and fine-textured soil. Five grams of cleaned soil were deployed in uncovered glass Petri dishes. Every sample was spiked with 125 µL of a stock solution containing 16 US EPA priority PAHs at a concentration of 100 µg/mL and exposed to different climate conditions in a climate chamber. In order to assess any potential changes in PAH concentration over time, soil samples were daily removed from the climate chamber the following days: 1, 2, 3, 4, 5, 6, 7, 14 and 28. Dark controls were also performed to assess slow sorption processes. The results showed that PAHs behaved differently according to their molecular weight, temperature, radiation and soil texture. A decreasing trend of PAH concentrations in both soils samples exposed to daylight was observed, being the loss rate higher in the climate change scenario. Low molecular weight PAHs are more influenced by volatilization and sorption, while photodegradation is more evident for medium and high molecular weight PAHs. Photocatalysis may enhance photodegradation processes in the fine-textured soil, possibly due to the presence of iron, silicon, manganese oxides as well as humic acids. Future studies will be focused on the potential formation of metabolites in the PAH photodegradation process, since some of them could be even more toxic than their parental compounds

381 Decomposition of Perfluorinated Alkyl Acids in Enzyme-catalyzed Oxidative Humification Reactions
Qiao, X. Tao, University of Georgia / Department of Crop and Soil Sciences

Perfluorinated alkyl acids (PFAAs) have drawn increasing concern due to their global distribution, extreme persistency and environmental toxicity. The unique molecular structure of PFAAs make them extremely resistant to degradation and, thus it still remains a significant challenge to remediate soil or waters contaminated by PFAAs with environmentally friendly approaches. We have conducted a series of experiments to examine the degradation of PFAAs in enzyme catalyzed reactions. Peroxidases and laccases were used as model oxidases, and perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) as model target chemicals. We have also conducted experiments in soil slurries with laccase as model mediator, and hydroxyl peroxoanionic acid (HPOA) and perfluorocarbonate (PFOS) as model target chemicals. We have also conducted experiments in soil slurries with laccase as model mediator, and hydroxyl peroxoanionic acid (HPOA) and perfluorocarbonate (PFOS) as model target chemicals. The results of our experiments demonstrated that PFAAs can be decomposed in ECOHR under environmentally relevant conditions in both aqueous phase and soil slurries. The findings suggest that ECOHR may provide a novel scheme for in situ remediation of sites contaminated by PFAAs.
Towards realistic and landscape based prospective ecological risk assessments: mapping variability and diversity

382 Summarising the information already available on mapping environmental and ecological variability across Europe
S. Sala, European Commission DG Joint Research Centre / Institute for Environment and Sustainability Sustainability Assessment Unit

383 Scientific needs for landscape risk assessment and ongoing work
E. Capti, Universitat Catolica del Sacre Cuore

V. Poulsen, ANSES / French Agency for Food Environmental and Occupational Health Safety

385 View on landscape risk assessment and scientific needs for covering European drivers
A. Alix, Dow Agrosciences / Risk Management

386 Landscape based ecological risk assessment, available methods and future outlook (science and fiction)
F. Streissl, EFS/A - Pesticides Unit; M. Arena, EFS/A - European Food Safety Authority / Pesticides; D. Auteri, Auteri / Pesticides Unit; M. Egsmose, EFS/A; A. Ippolito, EFS/A - European Food Safety Authority / Institute for Environment and Sustainability IES; R. Sharp, EFS/A - European Food Safety Authority / Pesticides Unit; C. Szentes, Pesticides Unit; J.V. Tarazona, European Food Safety Authority / Pesticides Unit

The presentation gives an overview on the advantages (and challenges) of moving from field based to landscape based risk assessments and spatial mapping of risk for pesticides. The potential use in risk assessment and risk management is highlighted. Important points for a spatially explicit risk assessment and risk mapping for the different regions in Europe are for example: the definition of specific protection goals for the landscape level and associated trigger values and risk assessment schemes. Furthermore, detailed maps (geo-referenced data) with a high resolution which include all important landscape elements and maps of distribution of species. Some of the data and tools are already available but still need to be developed. Examples from recent EFS/A opinions and guidance show how landscape based pesticide risk assessments could be developed in future.

387 Final discussion
J. Tarazona, EFS/A

Alternative approaches for ecotoxicity assessments (I)

388 Harmonizing the Harmonized Test Guidelines: Fish Developmental Biology Terminology and Animal Testing Implications
S.E. Belanger, The Procter & Gamble Company / Central Product Safety Dept; H. Sanderson, Aarhus University / Environmental Science; D. De Zwart, RIVM / Centre for Environmental Fate and Effects Division; P.W. Wilson, Sanofi U.S., Inc. / Health Safety and Environment

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The Threshold of Toxicological Concern (TTC) concept is well established for assessing human safety of indirect food additives and has been reapplied for a variety of endpoints including carcinogenicity, teratogenicity, and reproductive toxicity. The TTC can be used as a short-term screening-level risk assessment tool with potential for rapid decision-making, fully utilizing existing knowledge, reasonable conservativeness for chemicals used in lower volumes and reduction or elimination of unnecessary animal tests. TTC has found particular favor in the assessment of chemicals used in cosmetics and personal care products as well as other chemicals traditionally used in low volumes. Use of the TTC in environmental safety (termed an eco-TTC) is just beginning, with several recent meetings exploring the concept in various applications. Key questions focus on hazard extrapolation of diverse taxa across trophic levels, importance of mode of action, and whether safe concentrations for ecosystems estimated from acute or chronic toxicity data are equally useful and in what contexts. This platform will provide an overview of the theoretical basis for developing an eco-TTC with an initial exploration for chemical assessment and boundary conditions for use. An international collaboration under the ILSI Health and Environmental Sciences Institute has been established to address challenges related to developing and applying useful eco-TTC concepts.

*Disclaimer: The views, conclusions and recommendations expressed in this article are those of the author and do not necessarily represent views or policies of the
High Accuracy QSARs for REACH - The need to boost the implementation of Good Modelling Practice

A. Roncaglioni, Istituto di Ricerche Farmacologiche Mario Negri; F. Como, Istituto di Ricerche Farmacologiche Mario Negri

Under the EC Regulation 1107/2009 there is a requirement for registrants of novel chemicals, represented by circles. In the case of BCF the user can see the plot of the relationship between logBCF and logP. BCF and other available experimental data are also shown. The tool presented in this work has been designed to facilitate the application and visualisation of read-across methods. Its main advantage is the visual representation of the major evidences available to address a read across with similar compounds facilitating the application of this approach in a regulatory setting. The implementation of a similar approach to address acute fish toxicity and other endpoints is currently ongoing.

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Invertebrate models in environmental toxicology (I)

G.L. Ferreira, M.N. Flynn, LEAL Laboratory of Ecotoxicology and Environmental Microbiology School of Technology; G.d. Umbuzeiro, FACULTY OF TECHNOLOGY -UNICAMP / LEAL

The integration of population-level endpoints in the evaluation of toxicological effects of contaminants is considered of greater ecological relevance than the exclusive use of individual-level endpoints. Certain chemicals can affect demographic parameters at concentrations much lower than that indicated by traditional dose-response curve, resulting in population decline and extinction of populations. An offspring generated from ten Hyalella azteca couples, a cohort, was followed in laboratory conditions from birth to death of all individuals. From the cohort, life table was constructed and vital parameters obtained. The cohort monitored started with 77 individuals, declining until reaching zero individual, over seven months. The sex ratio was almost even, about 1.08, favorable to males. Reproductive potential of 26.89, generation time of 6.63 and intrinsic rate of increase (rm) of 0.49 were obtained. An alternative endpoint, instantaneous rate of increase (ri) is suggested as an alternative endpoint, easier to work since it is obtained from a partial life table. Toxicological sub lethal effects can be discerned through the use of referral of such parameters.

The development and application of crustacean biomarkers to monitor reproductive endocrine disruption.

S. Short, G. Yang, Y. Guler, A. Green Etxabe, University of Portsmouth; P. Kille, Cardiff University; A.T. Ford, University of Portsmouth / Biological Sciences

Despite the well documented cases of endocrine disruption in vertebrates and some molluscan groups, it is currently unknown whether reproductive endocrine disruption represents a threat to crustacean populations. The study of intersexuality provides an opportunity to study sex differentiation and sexual dysfunction in crustaceans. Crustacean intersexuality is associated with contamination and includes forms of sex reversal. In the case of BCF and other available experimental data are also shown. The tool presented in this work has been designed to facilitate the application and visualisation of read-across methods. Its main advantage is the visual representation of the major evidences available to address a read across with similar compounds facilitating the application of this approach in a regulatory setting. The implementation of a similar approach to address acute fish toxicity and other endpoints is currently ongoing.

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Invertebrate models in environmental toxicology (I)
will represent the first application of appropriate transcriptomic biomarkers to monitor feminisation and de-masculinisation in field populations of crustaceans.

396 Reproductive and physiological effects of neuro-active pharmaceuticals at low environmental levels in Daphnia magna

C. de Souza Machado, IDAE-CSIC Barcelona / Environmental Chemistry, B. Campos, IDAE-CSIC / Environmental Chemistry, C. Barata, CSIC / Environmental Chemistry

As the risks of emerging contaminants such as pharmaceuticals in the environment requires an understanding of their exposure regime and their effects at environmental relevant concentrations across species. Daphnia magna represents a frequent and abundant aquatic species to serve as an aquatic sentinel. Survival under starvation revealed a shorter life-span when compared to control. Energy reserves of daphnids were also altered as a consequence of the exposure to these compounds, revealing a shift in the energy allocation of the animals. Analyses of several gene transcripts by qPCR revealed de-regulation in their expression, giving some insights into the molecular mechanisms and MoA of these pharmaceuticals. Overall, this work highlights the use of integrated responses in D. magna, combining phenotypical and physiological and molecular endpoints, having great potential to elucidate MoA of emerging pollutants in the aquatic environment. Besides, our results provide consistent evidences on how measured reserve levels of these pharmaceuticals in the field are likely to be hazardous to aquatic biota.

397 An inverse U-shaped dose-response for toxicity of pesticide Dipel to Daphnia magna

A. de Souza Machado, Leibniz Institute of Freshwater Ecology and Inland Fisheries / Ecophysiology and Aquaculture; C. Zarfl, University of Osuna / Center for Applied Geosciences; W. Klos, Leibniz Inst. of Freshwater Ecology & Inland Fish.

Dipel is a worldwide used pesticide to control caterpillars in agricultural and urban areas. In most countries, however, there are no water quality criteria for this agent. Respirometry assays showed that the exposure to CBZ and PR significantly increased oxygen consumption, independently on the presence or absence of food, suggesting the involvement of energy expenditure. Survival under starvation revealed a shorter life-span when compared to control. Energy reserves of daphnids were also altered as a consequence of the exposure to these compounds, revealing a shift in the energy allocation of the animals. Analyses of several gene transcripts by qPCR revealed de-regulation in their expression, giving some insights into the molecular mechanisms and MoA of these pharmaceuticals. Overall, this work highlights the use of integrated responses in D. magna, combining phenotypical and physiological and molecular endpoints, having great potential to elucidate MoA of emerging pollutants in the aquatic environment. Besides, our results provide consistent evidences on how measured reserve levels of these pharmaceuticals in the field are likely to be hazardous to aquatic biota.

398 Obesogens beyond Vertebrates: Lipid Perturbation in the crustacean Daphnia magna


The increasing concern on emerging contaminants has urged for the need of novel ecotoxicological procedures in Environmental Risk Assessment. Current toxicity assays such as those based on acute and chronic responses may not be sensitive enough to detect emerging effects at low doses. Here we include data on a new assay developed in Daphnia magna juveniles to test obesogenic effects of contaminants. The bioassay is based on short term in vivo exposures to the tested chemicals and in vivo visualization of lipid reserves using the fluorescent dye Nile Red. The test was validated with a model pollutant known to be obesogenic, such as tributyltin (TBT), and other anthropogenic contaminants such as triphenylin (TPT), bisphenol A (BPA), 4-nonylphenol (NP), di-2-ethylhexyl phthalate (DEP), piriropyfen (PF), fenamidon (FEN), methoprene (MET), emamectin benzoate (EM), tebufenozide (TEB), as well as of natural hormones such as methyl farnosate (MF), 20-Hydroxyecdysone (20E) and ponasterone A (PA). The results obtained indicated that the Nile red bioassay was able to reproduce lipid reserve dynamics in D. magna within and between mating, reproductive events, juvenile life history studies were complemented with feeding, respiration and survival-stavration assays of exposed females and measurements of energy budget and gene expression. The selected pharmaceuticals were carbamazepine (CBZ), diazepam (DZP) and propranolol (PR), three widely prescribed compounds, already detected as considerable levels in the environment (ng to µg/L). Obtained results indicated that exposure to the studied compounds increased the offspring population but at different concentrations. Enhanced offspring production was food dependent, only occurring at limiting food conditions. Joint effects of tested ternary mixtures were additive and follow the concentration addition model, which may indicate that the studied compounds act on reproduction by similar MoA.

Respirometry assays showed that the exposure to CBZ and PR significantly increased oxygen consumption, independently on the presence or absence of food, suggesting the involvement of energy expenditure. Survival under starvation revealed a shorter life-span when compared to control. Energy reserves of daphnids were also altered as a consequence of the exposure to these compounds, revealing a shift in the energy allocation of the animals. Analyses of several gene transcripts by qPCR revealed de-regulation in their expression, giving some insights into the molecular mechanisms and MoA of these pharmaceuticals. Overall, this work highlights the use of integrated responses in D. magna, combining phenotypical and physiological and molecular endpoints, having great potential to elucidate MoA of emerging pollutants in the aquatic environment. Besides, our results provide consistent evidences on how measured reserve levels of these pharmaceuticals in the field are likely to be hazardous to aquatic biota.

399 High mutational rates of large-scale duplication and deletion in the environmental toxicology model, Daphnia pulex

N. Keith, Indiana University - Bloomington / School of Public and Environmental Affairs; A. Tucker, Southern Arkansas University; C. Jackson, Indiana University Bloomington; W. Sung, Indiana University; J. Lucas Lledo, Leibniz Institute of Freshwater Ecology and Inland Fisheries; D. Schirner, RUTGERS UNIVERSITY / Freshwater Ecology and Inland Fisheries; M. Jordão, CSIC / Environmental Toxicology; B. Pina, IDAEA / Environmental Toxicology

Knowledge of the rate and spectrum of genomic mutation is critical for understanding an organism’s interaction with its environment—including toxic environments; it provides the variation underlying their tolerance, susceptibility, and ability to adapt. Here, we utilize the microcrustacean Daphnia pulex, an established ecotoxicological model, to understand the rate and spectrum of genomic mutation. D. pulex is a sentinel species, being the primary grazer of algae and main forage of fish in lentic environments, and is an established model in toxicology, evolution, genetics, developmental biology, and ecology. Knowledge of mutational processes in the D. pulex genome therefore allows us to understand how Daphnia populations, and their corresponding communities, respond to anthropogenic stressors such as pollution and global warming, which have been found to have profound influences on ecosystem health, and are known to influence genomic mutation. Using a mutation-accumulation (MA) experiment, which through relaxing natural selection by the genetic bottlenecking of successive generations allows us to recover nearly all mutations that are not lethal or cause sterility, we find the rates of gene duplication and deletion are over an order of magnitude higher than an other organism, and are driven by large-scale mutations that spontaneously duplicate or delete over 100,000 DNA base pairs, simultaneously changing the count of hundreds of genes. Here, we find that gene conversion, which occurs during DNA repair of single- and double-strand breaks is rampant in D. pulex, and greatly contributes to genome composition.

Multistress in aquatic environments: the big picture (I)

400 Sub-lethal effects of acute salinity exposure on stream invertebrates: predation as a case study

M. Caneiro-Arques, University of Vic / Spain / BETA Technology Centre
Aquatic Ecology Group; G. Peixoto, FEM Research group University of Barcelona; M. Sala, Universitat de Barcelona / Freshwater Ecology and Management FEM research group; N. Prat, University of Barcelona, FEM Research Group / Ecology; M.D. Faria, CESAM, University of Aveiro / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; C. Barata, CSIC / Environmental Chemistry; B.J. Kefford, University of Canberra / Department of Environmental Science

Salinisation is a growing global threat, recognized as one of the main stressors to freshwater ecosystems. Yet, its effects on the trophic structure of rivers and streams are poorly understood. We conducted a mesocosm experiment to test if elevated conductivities can induce a stress response in the invertebrate predator Ephemeroptera resulting in its predation stimulated. The experiment was conducted at a flow-through mesocosm of 12 artificial stream channels relying on water pumped from a diversion channel of the Llobregat River (Barcelona, Spain). We used two treatments levels: sal (nominal conductivity = 6 mS cm$^{-1}$) vs. control (nominal conductivity = 0.6 mS cm$^{-1}$), and Ephemeroptera presence vs. Ephemeroptera absence. Biomarkers were used to stress the test response of Ephemeroptera to the salt treatment. Invertebrates were collected from two different substrates in the artificial streams: the stones and the gravel bed. Chlorophyll a bioassays were estimated as measure of primary production. Results showed that salinity affected significantly (P<0.05, Student t test) both antioxidant defenses and phase II detoxification metabolism, with reduced catalase activity and increased glutathione S transferase activity and induced oxidative stress measured as lipid peroxidation. These results thus indicate that the tested salinity levels could have detrimental effects on Ephemeroptera and reveal different effects of the ecosystems studied. We induced invertebrates to migrate from the stones to the gravel bed, probably looking for refugia. The salt treatment resulted in a reduced invertebrates’ density both in the absence and presence of predators, but only for the communities attached to the stones. Our results suggest that the individuals burrowing in the gravel beds were less exposed to salt toxicity. Chlorophyll a (chl a) concentrations were highest in the control treatment (nominal salinity a = 26.6 mg m$^{-3}$) and lowest in the salt treatment with no predators (mean chl a = 7.9 mg m$^{-3}$). The channels in which Ephemeroptera were added registered intermediate chl a concentrations (mean chl a = 17.9 and 14 mg m$^{-2}$; no salt and salt treatments respectively). This suggests that sub-lethal effects of salinity on Ephemeroptera propagated to ecosystem functioning.

401 Does mussel nutritional status act as a confounding factor on biomarkers responses? C. González-Fernández, Spanish Institute of Oceanography / Marine ecosystems; S. Díez, Concepción / G.P. and G.P. / Centro Oceanográfico de Murcia; J. Campillo, Instituto Español de Oceanografía; L. Vinas, Spanish Institute of Oceanography; A. Franco, Instituto Español de Oceanografía; J. Bellas, Centro Oceanográfico de Vigo / IEO (Instituto Españo del Oceanografía)

Large scale marine monitoring programs have some difficulties in the assessment of the effect of pollution in the biological responses of the organisms, mainly due to the complexity of the biological processes. Two main aspects of the invertebrates were investigated: how invertebrates migrate from the stones to the gravel bed, and probably looking for refugia. The salt treatment resulted in a reduced invertebrates’ density both in the absence and presence of predators, but only for the communities attached to the stones. Our results suggest that the individuals burrowing in the gravel beds were less exposed to salt toxicity. Chlorophyll a (chl a) concentrations were highest in the control treatment (nominal salinity a = 26.6 mg m$^{-3}$) and lowest in the salt treatment with no predators (mean chl a = 7.9 mg m$^{-3}$). The channels in which Ephemeroptera were added registered intermediate chl a concentrations (mean chl a = 17.9 and 14 mg m$^{-2}$; no salt and salt treatments respectively). This suggests that sub-lethal effects of salinity on Ephemeroptera propagated to ecosystem functioning.

402 Sensitivity of Pacific oyster embryos (Crassostrea gigas) to combine effects of pollutants and salinity P. GAMAIN, EPOC; P. Gonzalez, Géochimie et Ecotoxicologie des Mêtaux dans les systèmes Aquatiques GEMA team UMR CNRS Université Bordeaux; J. Cachat, Université Bordeaux / EPOC; B. Morin, EPOC

The Pacific oyster represents the world’s aquaculture production, with 4.6 million tons produced in 2006. France is the fourth world producer of oysters and Crassostrea gigas is the principal cultivated species in the Arcachon bay. However in recent years, problems of recruitment, capture and mortality of juvenile and adult oysters occurred. The increased frequency of these events can be indicative of changes in the global quality of the Arcachon bay. Previous studies have highlighted the presence of many plant molecules, with significant concentrations in water, mainly herbicides such as metolachlor and its metabolites, and “anti-fouling” as copper. Due to global warming, the Arcachon bay is facing other environmental problems such as increase of surface water temperatures and/or salinity fluctuations. Previous studies have highlighted that early life stages of marine invertebrates are sensitive to environmental changes. Therefore, in the context of global climate change, vulnerability of D-larval stages of C. gigas to salinity and pollutants was investigated. Embryotoxicity bioassay has been used. Male and female oysters of a commercial hatchery were induced to spawn by thermal stimulation. Eggs were fertilized with sperm to obtain embryos. Effects of copper (0.5 µg L$^{-1}$, 1 µg L$^{-1}$ corresponding to environmental concentrations, 10 and 50 µg L$^{-1}$) and metolachlor (10 ng L$^{-1}$ corresponding to environmental concentration, 100 and 1000 ng L$^{-1}$) on the early life stage of Pacific oyster D-larvae were examined at various salinities (18 ; 21 ; 24 ; 27 ; 30 ; 33 ‰). After 24h, metabolic abnormalities of D-larvae were analyzed. Significant increases of the percentage of abnormal D-larvae were observed from 0.5 µg L$^{-1}$ of copper and 10, 100 ng L$^{-1}$ of metolachlor. The current study suggests that copper, and metolachlor can induce larval abnormalities in a population of exposed oysters at environmentally relevant concentrations. The low feasibility limit of the embryo-larval test is around 24 % of salinity. With copper, at all concentrations tested, salinity does not impact the percentage of larval abnormalities whereas, with metolachlor, larval abnormalities increase significantly with decreasing salinity.

403 Biotransformation of selected azole fungicides in the aquatic invertebrate Gammarus pulex and their effects in mixture on oxidative drug metabolism A. Rösch, Eawag / Environmental Chemistry; S. Anlker, Eawag Swiss Federal Institute of Aquatic Science and Technology / Environmental Chemistry; J. Hollender, Eawag / Environmental Chemistry

Xenobiotics, such as pesticides and pharmaceuticals are distributed ubiquitously in the aquatic environment and their co-occurrence is often reported. One class of fungicides are azoles including triazoles and imidazoles. They act by inhibiting the cytochrome P450 (CYP) reductase and target organellar oxidoreductases (CYPs). These results thus suggest that copper, and metolachlor can induce larval abnormalities in a population of exposed oysters at environmentally relevant concentrations. The low feasibility limit of the embryo-larval test is around 24 % of salinity. With copper, at all concentrations tested, salinity does not impact the percentage of larval abnormalities whereas, with metolachlor, larval abnormalities increase significantly with decreasing salinity.

404 Physiological responses in freshwater bivalves to microcystin-LR and Roundup Flash®

C. Wiegang, University of Southern Denmark / Department of Biology; M. Götzé, University of Southern Denmark; M. Malecot, Université Rennes / UMR Ecobio; B. Guevel, C. Pineau, Proteomcs Core Facility Biogenouest / IRSET / Inserm U 740 Lakes, ponds and rivers are often exposed to fungicides and fertilizers. Excess plant nutrients are drivers for cyanobacterial blooms, which pose a natural environmental risk due to their capacity to produce toxic metabolites. The cyanobacterial toxin microcystin-LR binds and inactivates protein phosphatase of type 1 and 2A, and may cause death of the organism. Within exposure
Concentration and duration limits, microcystins can be detoxified via conjugation to glutathione by glutathione S-transferase (GST). Glyphosate is one of the most used herbicides worldwide, it inhibits the amino acid synthesis in plants through the shikimic acid pathway. Evidence about its harmful effects in non-target organisms is growing, in particular for the commercial formulations. Freshwater mussels are sessile organisms and via their filtration for food exposed to huge quantities of water. Whereas some indigenous species densities decline, invasive ones proliferate in the same scenario. The present study aims to evaluate the concentration of four Main text in this section resumes above and continues from here. This result could be related to their lower thyroid activity but also to the impairment of the HPT axis was also investigated. In yellow individuals, high FT₃/FT₄ ratios and TR₁ (brodifacoum, flocoumafen, difethiolone) have been restricted to indoor use to limit potential secondary poisoning of non-target wildlife. Monitoring the impact of new UK stewardship on SGAR exposure in non-target wildlife R.F. Shore, Centre for Ecology & Hydrology (NERC); P.A. Henrys, Centre for Ecology and Hydrology; L.A. Walker, Centre for Ecology & Hydrology Residues of second-generation anticoagulant rodenticides (SGARs) have been detected in non-target wildlife (i.e. species blood Pb levels) and in around buildings. Open area use is also likely to be authorised for some compounds. These changes will require compliance with a stewardship scheme designed to enhance best practice and reduce non-target exposure. These changes in authorisation may alter exposure and risk to non-targets, but the direction of change may depend on the effectiveness of and compliance with stewardship scheme designed to enhance best practice and reduce non-target exposure. How can any such changes be measured? The Preatory Bird Monitoring Scheme (PBMS – http://pbms.ceh.ac.uk) has monitored liver SGARs in barn owls (Tyto alba) for more than 20 years. This large, unique dataset has provided a baseline against which the effects on non-target exposure changes in authorisation can be judged. We analysed our baseline data to determine the sensitivity in which changes in SGARs can be detected. The liver residue data for individual compounds and summed SGARs were not normally distributed, necessitating that we split the data into two normally distributed groups for each compound (< 100 ng/g and > 100 ng/g wet wt). These groups can be considered “background” and “toxically significant” respectively. We predict that analysis of 100 birds/yr should enable detection of changes of 20% from baseline for individual compounds within 1-4 years (95% confidence limits) and a change of 50% in summed SGARs within a year. Such changes, together with consideration of the proportion of birds with “background” and “toxically significant” residues, can be used as potential target or trigger values to determine the impact of stewardship and if further changes to authorisations are needed, respectively.

**407** Factors affecting blood lead (Pb) concentration in griffon vultures: a spatially explicit correlation

P. Mateo-Tomás, Instituto de Investigación en Recursos Cinegéticos (IREC) CSIC-UCLM-JCCM; P.R. Camarero, I.S. Sanchez-Barbudo, UCLMCSCIC; P.P. Olae, Instituto de Investigacion en Recursos Cinegéticos; R. Mateo, U.C.L.M.-C.S.I.C / Instituto de Investigacion en Recursos Cinegéticos Lead (Pb) toxicity poses a significant threat for the conservation of many species. We determined Pb blood exposure in griffon vultures (Gyps fulvus) by assessing blood Pb concentration (ng/ml) from 691 individuals captured in 2008-2012 at 27 feeding points in Aragón, NE Spain. We performed (generalized linear and cumulative link) mixed models to identify the main factors influencing blood Pb concentration. This information was further used to produce spatially explicit maps of Pb exposure for the species. Almost half of the sampled vultures (i.e. 44.9 %) were exposed (i.e. blood Pb concentration >200 ng/ml) was the main variable explaining vulture Pb exposure. Poultry supplies to feeding points negatively influenced blood Pb concentration, which was higher in males than in females. Vulture Pb exposure seems to be higher in winter than in summer, probably responding to a higher presence of additional lead sources (e.g. big game hunting remains) in autumn and winter. The high prevalence of Pb-exposed griffon vultures in the study area (44.9%) seems to be mostly due to soil Pb bioavailability favouring Pb presence in vultures’ main food resources (i.e. livestock and wild animals). This elevated blood Pb background can increase the effects of additional Pb exposure like Pb amonitons from game carcasses. Managers can take advantage of the maps here produced to identify those areas under higher risk of Pb exposure for vultures (and other scavengers) and take action to limit exposure to potential Pb sources (e.g. big game hunting remains).
ChronicEnvironment; S. Drouhot, UMR 6249 Chrono-environnement; B. Valot, University of Franche-Comté/CNRS / ChronoEnvironment; F. Mora, Conservatoire Botanique National de Franche-Comté - Observatoire Régional des Invertébrés; T. Cornier, Centre régional de phytosociologie agréé Conservatoire Botanique National de Bailleul; R. Scheiffer, University of Franche-Comté / ChronoEnvironment

Trace metal contamination can deplete energy and matter as well as the transmission of pollutants and pathogens within food webs. There are studies demonstrating that the number of trophic levels (“vertical” diversity) is a key factor in the bio-magnification of some pollutants along the food chains, but the importance of community diversity within a given trophic level (“horizontal” diversity) on the transfer of contaminants to a higher trophic level is still an open question. The aim of this study is to correlate the presence of trace metals in the tissues of the wood mouse (Apodemus sylvaticus) to the resource community diversity both in the field and in the diet of the animal. Sampling took place in the surroundings of the former Metaleurop Nord Pb and Zn smelter in northern France. In 500m x 500m sampling squares vegetation was quantified and mapped, invertebrate fauna was sampled using pitfall traps and colored plates and small mammals were trapped using small break-back traps. The stomach contents (SC) of the rodent were extracted and used for quantification of TM by ICP-AES/MS and for molecular identification of food items by using DNA metabarcoding. Primers were chosen to amplify plant, mollusc, arthropod, earthworm, and fungus DNA. Exposure to Cd, Pb and Zn was significantly lower in September than in April. The taxonomic richness of both plant and invertebrate food items in SC was significantly higher in September than in April, and of different trophic levels. The diet composition changed with the season (April: 34% herbivory and 66% carnivory; September: 38% herbivory and 62% carnivory). Significant but weak relationships between TM concentrations in SC and food item richness in SC were detected in April for Pb (with both plant and invertebrates richness) and for Cd (with total food item richness). No correlation was detected in September and for Zn. Our results suggest that the link between biodiversity and exposure is TM-specific and not straightforward. Further analyses will focus on resources’ life history traits related to TM accumulation capacity.

410 Can long-term exposure to trace metals (Cd, Pb) induce fluctuating asymmetry in wild wood mice (Apodemus sylvaticus)?

N. Tede, University of Franche-Comté / ChronoEnvironment; F. Muñoz-Muñoz, Universitat Autònoma de Barcelona / Departament de Biologia Animal; C.C. Fritsch, CNRS / UMR ChronoEnvironment; R. Scheiffer, University of Franche-Comté / ChronoEnvironment; A. Sánchez-Chardi, Autonomous University of Barcelona / Microscopy Facility

Trace metal contamination can have deleterious effects on foetal health by altering growth, development, and the target organs of the individual. This study calculates the fluctuating asymmetry (FA), defined as subtle random deviations from perfect bilateral symmetry, which has been proposed as a quantitative biomarker to detect stress during the development period. In this context, the aim of the present work was to evaluate the effect of long-term TM contamination on developmental stability of a wild population of wood mouse (Apodemus sylvaticus). The field work took place in the surroundings of the former Pb and Zn smelter in northern France. After more than 100 years of activity, this area is drastically polluted, mainly by Pb and Zn. Categories of environmental contamination (control, lightly, moderately and highly polluted) were determined depending on Cd concentrations in soils. In spring 2011, 61 mice were live-trapped along a pollution gradient of contamination. Metal concentrations (Cd, Pb) in soft tissues (liver and kidneys) were measured and analyzed. To determine shape FA, 17 two-dimensional landmarks were digitized on each right and left mandible. Histological alterations attributable to chronic TM exposure were also assessed in the liver and the kidneys of mice. Results indicated an important transfer from the environment to the biota. Concentrations of Cd and Pb in the liver increased along the gradient of contamination (F = 18.1, df = 3 and 57, p < 0.001 for Cd and F = 9.4, df = 3 and 57, p < 0.001 for Pb). Preliminary results indicate a trend for size FA (F = 1.8, df = 3, p = 0.157, Fig. 2) but not for shape FA (0.263 < p < 0.811) to increase along the gradient of contamination. No correlation could be established between TM concentrations in soils or in target organs and FA shape or size (0.162 < p < 0.851). Size FA correlated with the general state of the kidneys in mice (Spearman’s rank correlations r = 0.27, p = 0.036) and was more specifically with the severity of necrosis in the kidney tissues (Spearman’s rank correlations r = 0.34, p = 0.008). Shape FA was also related to the severity of fibrosis in kidneys (Spearman’s rank correlations r = 0.29, p = 0.026). These preliminary results show that measures of developmental instability are less sensitive to detect TM pollution impact on wood mice than other biomarkers quantified such as the histopathological evaluation.

411 Imaging of structural and ultrastructural effects of chronic exposure to Pb and Cd in liver of wood mouse, Apodemus sylvaticus: qualitative and quantitative approaches

A. Sánchez-Chardi, Autonomous University of Barcelona / Microscopy Facility

Chronic exposure to non-essential trace metals (TM)s produces toxic effects in target tissues and cells that may be detected by morphological changes at structural and ultrastructural levels. TM$s induce toxic effects in liver that may be observed on the entire organ or in its cells such as changes in volume and size, inflammatory and carcinogenic processes, cell death, cell degeneration, nuclei damage, or mitochondrial impairment among others. In the present study, we compared for the first time the damages of chronic exposure to different environmental levels of lead (Pb) and cadmium (Cd) in wood mice, Apodemus sylvaticus, at tissue (hepatic tissue) and cell (hepatocytes) levels using qualitative and quantitative approaches. These morphological quantifications were compared with TM levels in liver as biomarkers of effect in wood mice as a sentinel species of environmental pollution. In spring 2011, 84 wood mice were live-trapped around the former smelter. TM concentrations (Cd, Pb) in liver were measured in each individual by furnace atomic absorption spectrometry and expressed as mg/kg dry mass. The alterations attributable to metal exposure in hepatic samples were analyzed using both conventional techniques of light microscopy and transmission electron microscopy. The concentrations of Cd and Pb in the liver differed significantly along the gradient of contamination. The qualitative and quantitative approaches at tissue and cellular levels showed increases of frequency, extension of lesions and severity of several hepatic alterations related to chronic exposure to TM$s. More specifically, the necrosis and cell degeneration of hepatocytes were correlated with both the concentrations of Cd and Pb in the liver. The qualitative and quantitative approaches at cell level in healthy hepatocytes revealed an increase of glycosgen accumulation and mitochondrial damage in mice from the polluted sites. These preliminary results point out in the important metabolic effects of TM$s exposure in two crucial functions for cell energy as the storage of glycosgen as rapid energy source and by cellular respiration in mitochondria. The disturbances in crucial cell functions of liver, the main target organ for many pollutants including TM$s, detected in mice exposed to Pb and Cd, indicate relevant toxic effects that may have a deleterious impact on physiological functions of wild individuals exposed to anthropogenic stressors.

Innovations in environmental analytical chemistry: the quest for pollutants using target and non-target approaches (I)

412 Development and validation of a LC-MS/MS method for the determination of surfactants in wastewater treatment plant using QuEChERS and solid phase extractions

A. Bergez, Institut des Sciences Analytiques; B. Giroud, L. Wiest, Institut des Sciences Analytiques UMR TRACES Team; E. Vuilliet, CNRS

Because of their specific physicochemical properties (amphiphilicity, solubility in polar and non-polar liquids, ability to form micelles, etc) surfactants are widely applied in industry and in the household. One of the main pathways of their introduction in the environment is their rejection by treatment plants. As their applications are on a very large scale, it has therefore become necessary to reach a more detailed understanding of their environmental fate. The analysis of complex matrix such as effluents or sludges needs a rigorous sample preparation to obtain repeatable and selectable analysis. The usual techniques are liquid–liquid extraction (LLE), solid phase extraction (SPE), or accelerated solvent extraction (ASE). Additionally, innovative techniques such as liquid–liquid extraction assisted by solid phase extraction (QuEChERS) may be used. Nowadays, high-performance liquid chromatography (HPLC) is usually coupled with a mass spectrometer detector (MS) (or tandem mass spectrometry detector MS–MS), what allows for detection, identification and quantification of the various compounds in various environmental matrices. This works describes particularly the separation and determination of several classes of surfactants as quaternary ammoniums, betains, alkylphenol ethoxylated, linear alkylbenzene sulphonates, sodium sulphates, and alcohol polyethoxylated using tandem mass spectrometric detection. This work leads to innovative and validated analytical methods to detect and estimate several classes of detergents in wastewaters and solid matrices. This work has confirmed, first and foremost, that surfactants are indeed highly concentrated in urban environment. Although biological process removed a significant proportion of the total pollution, concentrations in final effluents remain significant, up to 100 µg/l. The coupling of a powerful analytical method with the QuEChERS extraction achieved the necessary selectivity and sensitivity to the detection of these compounds at low levels, below the ng/l.

413 Levels and fate of antifungal pharmaceuticals at sewage treatment plants

J. Casado Agrelo, Analytical Chemistry; G. Castro, University of Santiago de Compostela; I. Rodríguez, University of Santiago de Compostela / Department of Analytical Chemistry Institute of Food Analysis and Research; M. Ramil, R. Cela, University of Santiago de Compostela

Antifungal drugs are a broad group of drugs recognized as emerging environmental contaminants. These pharmaceuticals are active against infections caused by fungi and they are suspected of being endocrine disruptors. In this work we present the optimization of improved selectivity extraction and enrichment methods for the analysis of antifungal drug residues in water and sludge samples using a liquid chromatography quadrupole time-of-flight mass spectrometry system. Analytes extraction and concentration from water samples was performed using a mixed-mode (reversed-phase and cationic exchanger) solid-phase extraction cartridge followed by a sequential elution protocol. As a result, the efficiency of electrospray ionization was similar for calibration standard solutions and sample
extracts, even in case of raw wastewater. Other variables related with the efficiency of the extraction step, such as sample pH and potential losses during sample filtration, were also optimized. Improving the performance of previous sample preparation methods, and attaining limits of quantification low enough to deal with environmental samples. Regarding sludge samples, an effective and selective, modular sample preparation method for the extraction of the pharmaceuticals was proposed. It comprises the pre-treatment of the solid samples by a maceration step, followed by a stirring-on-ice-concentrated with a cationic exchanger SPE cartridge. In comparison with previously published sample preparation methodologies, the developed approach greatly simplifies sample handling and reduces attainment of ESi ionization for sample extracts when compared to standard solutions. The obtained absolute recoveries ranged between 75 and 117% in water samples, and between 85 and 119% in the case of sludge samples. Limits of quantification of the method varied between 2 and 15 ng L⁻¹ in water samples, and between 5 and 8 ng g⁻¹ in the case of the sludge samples. Fluconazole, ketoconazole, miconazole and clotrimazole were measured in wastewater samples at concentrations up to 200 ng L⁻¹, while azoles, ketoconazole, clotrimazole and miconazole were ubiquitous in urban sludge samples, with maximum average concentrations (above 400 ng g⁻¹) corresponding to clotrimazole.

414 High-resolution mass spectrometry approaches for tracking down transformation products of diclofenac and structurally related non-steroidal anti-inflammatory drugs (NSAIDs) in wastewater
S. Perez, IDAEA; T.H. Miller, University of Bordeaux / UMR EPOC Equipe LPTC; P. Pardon, University of Bordeaux / UMR EPOC 5805 EPOC; L. Barron, Kings College London / Analytical and Environmental Science

Over the last five years, liquid chromatography-high resolution mass spectrometry (LC-HRMS) has emerged as a promising technique for qualitative methods. HRMS can provide a great deal of information from the full-scan acquisition allowing the confirmation of the identity of each analyte using accurate mass and isotope cluster of the molecular ion. Of particular value, however, is mass spectral information on fragment ions which can be generated using platform-specific functions. Therefore, this technique can be of great interest for the application of new approaches for the identification of transformation products (TPs) of polar compounds such as non-steroidal anti-inflammatory drugs (NSAIDs) in environmental samples. NSAIDs are a chemically heterogeneous large group of drugs used primarily to treat inflammation and fever. They reach the WWTPs after their use in human medicine. Compared to the data dealing with the fate of NSAIDs in the environment, very little evidence has been published as regards metabolic pathways in complex microbial communities like those encountered in tank of the activated sludge treatment. In a previous work, in the same set-up related in this work, we have tentatively identified nitroso (TP324) and nitro (TP340) derivatives of diclofenac (DCF). The current work intended to investigate still unexplored aspects in the environmental fate in WWTP of the NSAIDs related to DCF such as meclofenamic acid, flufenamic acid, tolkenamic acid, and lumiracoxib identifying their TPs. By applying HR-MS in the analysis of complex samples, the detection and identification of the TPs was greatly facilitated: following the assignment of elemental formulæ based on exact mass, relative isotope abundances and isoeppe spacing of characteristic product ion spectra acquired through the QExactive allowed to identify the most likely sites of structural modifications in the TPs. Biodegradation experiments in batch-reactors loaded with mixed liquor demonstrated that similar degradation profiles were observed for the related compounds of DCF. In the batch reactors, we have reported the discovery of unusual microbial nitro/nitrosation TPs of related NSAIDs of DCF. These results are of potential interest with regard to environmental pathways through biological reactions to generate these TPs in lab-scale reactors and also with the first evidence of the occurrence of these TPs in WWTPs, reported in other work of our group.

415 Biogenic, non-extractable residues of pesticides
C. Possberg, RWTH Aachen University / Institute for Environmental Research Biology V; B. Schmidt, RWTH Aachen University; M. Telscher, Bayer CropScienceEnvironmental Fate / DevelopmentEnvironmental Safety; D. Schaefer, Bayer CropScience / Environmental Safety Environmental Fate; A. Schmets, Bayer CropScience / Synthetic Biology and Environmental Research. After the application of pesticides, residues are formed in plants and soil, which cannot be extracted with organic or inorganic solvents without destruction of the matrix and/or the chemical compound. While quantification of non-extractable residues (NER) is feasible by use of radioactively labelled compounds, direct analysis of the chemical nature of NER is not possible. Because microorganisms use a large number of xenobiotics as carbon and energy sources, the transformation and mineralisation of xenobiotics takes place in microbial communities. In this study, we determined the 
¹³C-labelled carbon atoms may be incorporated into microbial biomass. After cell death, dead biomass is incorporated in SOM (soil organic matter) thus forming biogenic NER (bioNER) which is unlikely to pose any risk to the environment. The formation of bioNER is not yet measured routinely in environmental fate studies of xenobiotics due to a lack of methodology (Kaeser et al, 2014). This investigation focusses on the use of proteinaceous carbon analysis because proteins make up the largest proportion of the bacterial cells, namely 50-55% of the microbial mass. The main goal of the experiments was to develop a rapid and reproducible method for quantifying bioNER based on “C-analyses”. Amino acids are known as microbial markers, which represent most of the analytically detectable biogenic components of SOM. Quantification of the magnitude and kinetics of the formation of biogenic residues during microbial degradation of the model substance bromoxynil in soil was examined. The 
¹³C-distribution within the system was analysed in order to establish a mass balance comprising CO₂ formation, solvent-extractable residues, total NER and bio-NER. In soil treated with 
¹³C-bromoxynil after 57 days of incubation significant amounts (approx. 9 %) of the applied 
¹³C were mineralised over time until up to 100% of the applied radioactivity. For further characterization of the NER fraction after extraction amino acids were separated by two-dimensional TLC and visualised by reaction with ninhydrin. Radioactive marked amino acids which have been formed during incubation were visualised by bioimaging and assigned to the corresponding reference amino acids. In addition the distribution of amino acids was analysed by GC-MS and HPLC-fluorescence/radiodetection. Kastner M, Nowak KM, Miltner A, Trapp S, Schäffer A (2014), Critical Reviews in Environmental Science and Technology 44, 2107-2171

416 Pharmaceuticals in the freshwater invertebrate, Gammarus pulex, determined using pulsed liquid extraction, solid phase extraction and liquid chromatography-tandem mass spectrometry
T.H. Miller, Kings College London / Analytical and Environmental Sciences; G. McEnvoy, Dublin City University; R.J. Brown, wca consulting; S.F. Owen, AstraZeneca / Safety Health Environment; N.R. Bury, Kings College London / Analytical and Environmental Sciences

Pharmaceuticals and their metabolites are continually discharged into aquatic environmental compartments via sewage treatment plant effluents leading to a pseudo-persistent exposure of aquatic life. These compounds may have adverse effects on reproduction, development and behaviour in biota. As water is the most important reservoir of pharmaceuticals, the risk of exposure to contaminated waters will remain in a constant flux thus highlighting the importance to determine internal concentrations for environmental risk assessment (ERA)². However, determination of pharmaceutical and personal care products at trace levels in biota remains analytically challenging. Herein, we present a new analytical method for the confirmation identification and quantification of 10 pharmaceuticals in the aquatic invertebrate, Gammarus pulex. Collected Gammarids were weighed, freeze-dried at -50 °C and homogenised before extraction in 5 mL acetonitrile in a ball-mill extraction vessel. Extracts were then purified and concentrated by solid phase extraction using a Waters Oasis HLB sorbent (200 mg, 6 mL barrel). Extracts were analysed using an optimised reversed-phase liquid chromatography-tandem mass spectrometry method in positive and negative electrospray ionisation modes. Method performance data is also presented herein showing acceptable linearity (R² > 0.98; range from 10 ng/g to 10 µg/g dry weight in most cases), reproducibility (RSD ≤ 20 %) and limits of quantification (4-45 ng/g). This method was successfully applied to the trace determination of several pharmaceuticals present in G. pulex across multiple river locations in the South London catchment. The occurrence of polar compounds such as antibiotics or antidepressants was found in most samples. Other variables related with the efficiency of the extraction step, such as sample homogenisation, extraction time, solvent type, internal standard and concentration, were also investigated. We conclude that the extraction method presented is robust, sensitive and rapid for the analysis of pharmaceuticals and personal care products in biota. Future research should be focused on the extraction of additional pharmaceuticals or contaminants which represent most of the analytically detectable biogenic components of SOM. Thus, the first evidence of the occurrence of these TPs in WWTPs, reported in other work of our group.
precipitation with acetonitrile, followed by centrifugation, filtration of extracts, and dilution with HPLC-grade water. Method optimization was conducted with the use of experimental designs and included study of the protein precipitation solvent, influence of pH, nature of the on-line SPE cartridge, on-line SPE loading flow rate and loading volume. The method was validated by assessing instrumental and method detection limits, linearity range, as well as precision, recovery, accuracy and stability. Environmentally relevant limits of detection were reported (0.0001–0.13 ng.g⁻¹) and 17/23 out of the 23 target analytes, 17 showed excellent accuracies (±20%). The analysis of a human serum certified reference material (NIST SRM 1957) was also included to evaluate method accuracy. The optimized method was subsequently applied to a selection of Antarctic seabird species, 11/23 targeted analytes being detected (ZEPASs range: 0.15–41.9 ng.g⁻¹). The reduced plasma sample size required (25 µL) implies that this method could also be applied to the analysis of PFSAs in small bird and fish species.

**Challenges in Wastewater Treatment and Reuse and the Agricultural Use of Manures and Biosolids (I)**

**418**

**Implementation of the Swiss water protection act: results from the first mover with advanced wastewater treatment**

C.S. Mc Ardle, Eawag / Department of Environmental Chemistry; M. Boehler, Eawag / Department of Process Engineering; E. Borowska, Silesian University of Technology / Department of Environmental Biotechnology; M. Bourgin, Eawag Swiss Federal Institute of Aquatic Science and Technology; J. Fleiner, Eawag / Process Engineering; J. Hollender, Eawag / Environmental Chemistry; C. Kienle; R. Teichler, Eawag / Environmental Chemistry; A. Wittmer, Process Engineering Swiss Federal Institute of Aquatic Science and Technology EAWAG; H. Siegrist, Eawag / Process Engineering

In March 2014, the new Swiss water protection act was accepted by the federal and national councils of Switzerland, and its implementation is planned for 2016. It was decided that measures will be taken at the municipal wastewater treatment plants to reduce the load of micropollutants entering the aquatic environment from the plants by about half. To reach this aim, about 100 out of the 700 Swiss wastewater treatment plants (WWTPs) will need to be upgraded with advanced treatment. The technologies proposed are ozonation or treatment with powdered activated carbon. The effectiveness of the measure should be evaluated by measurements of a set of substances which need to be eliminated by 80%. The Neugut plant in Dübendorf is the first WWTP in Switzerland where a full scale advanced treatment of wastewater with ozone has been installed and is running since March 2014. Eawag supported the installation of the plant scientifically and several investigations are currently running on the plant. The effectiveness of the treatment technology is assessed for chemical and for ecotoxicological quality control, and the production of transformation products formed in ozonation is investigated. Moreover, appropriate control and operation strategies are studied. Within this presentation, background information will be given on the new Swiss water protection act and results of the investigations at WWTP Neugut are shown.

**419**

**Preparing for Phase 2 of the Chemical Investigations Programme in England and Wales**

V. Jones; M. Gardner, Atkins Ltd; B. Ellor, UKWIR

The treatment and removal of trace substances from wastewater represents a significant challenge. In response to this challenge, a large monitoring programme was set up collaboratively by water companies in the UK - the Chemical Investigations Programme (CIP). Upon completion of CIP in 2013, it became clear that additional investigations were necessary. The CIP Phase 2 (CIP2) will focus on site-specific issues in England and Wales and the development of novel technologies to address these. This paper describes the complex process of scoping the scale and extent of this significant programme, including the establishment of effective communication channels between the project stakeholders, determining the overall objectives of the work, prioritising the substances of concern and designing the sampling. Approximately 600 wastewater treatment works (WWTWs) which contain specific sewage treatment plants (SSTPs) and WWTPs will be targeted. Influent, effluent, sludge and in-river samples will be collected. The analytical suite includes metals, priority substances, sanitary detergents, steroids, and pharmaceuticals. A key output of the scoping phase was the development of bespoke data processing tools, with an emphasis on effective graphical presentation of the results, accessible to the non-specialist. With the scoping now complete, CIP2 sampling is set to commence in early 2015. This ambitious programme will enable UK WWTWs to fulfills its obligations with regards to the Water Framework Directive and provide results of global importance on the sources and treatment of trace contaminants in wastewater and rivers.

**420**

**Grand Designs for Wastewater Recycling in Antarctica - Challenge of Micro-contaminant Assessment**

M. Allinson, The University of Melbourne / School of Chemistry; K. Kadokami, The University of Kitakyshu; D. Nakajima, National Institute for Environmental Studies; P. Scales, The University of Melbourne; G. Allinson, RMIT University / CAPIM; A. Knight, The University of Melbourne; J. Zhang, Victoria University; M. Packer, Australian Antarctic Division; K. Northcott, Veolia; V.J. Pettigrove, The University of Melbourne / Zoology; S. Gray, Victoria University

The Australian Antarctic Division (AAD) operates Australia’s Davis Station in the Antarctica. In 2005, Davis Station’s wastewater treatment plant failed, and since then untreated, micropolluted effluent has been discharged to the ocean. Although disposal of the station’s effluent by this method meets the minimum requirements specified by international agreements, an environmental impact assessment has identified that there is a clear a need for enhanced sewage treatment. A new advanced treatment plant that is small enough to fit inside two shipping containers has been designed and built by Victoria University, the University of Melbourne, the Australian Antarctic Division (AAD) and Veolia, and is now being installed by the Australian Water Recycling Centre of Excellence, to provide the enhanced sewage treatment. In the plant, secondary treated wastewater will undergo a multi-barrier process involving ozonation, ceramic microfiltration, biologically activated carbon filtration, reverse osmosis, ultraviolet disinfection and chlorination to remove trace organic contaminants and pathogens. This project will collect samples at each of the stage in the multi-barrier process, and use integrative bioassay methods (yeast-based recombinant reporter gene biosassay) to assess the performance of each of those barriers in removing organic chemicals of similar receptor binding properties and to assess the quality of the brine concentrate for disposal. To cost-effectively screen the widest possible range of micro-pollutants, the project will use the AQIS (Automated Identification and Quantification System) database GC-MS and LC-TOF-MS screening methods (systems that can determine more than 1200 organic chemicals). Once the performance of the system has been verified, the plant will be shipped to Antarctica and installed alongside a new secondary treatment plant. The combination of the two treatment plants will convert the station’s effluent into some of the cleanest water in the world which, when discharged to the ocean, is expected to have minimal impact on the local marine environment.
became completely embedded inside polymeric fibers. The particle dispersion was better for lower loadings, for which the relative amount of metal released to culture media was also higher. The microorganisms used in this study were *Pseudomonas putida* and *Staphylococcus aureus*. The antimicrobial activity of composite mats was assessed using SEM images, fluorescence microscopy, direct plate reading of fluorescent stains and plate count of colony forming units, among other. SEM images showed a higher sensitivity of *P. putida* to the inclusion of cobalt in fibers with a reduction in the number of viable cells reaching 40% with respect to neat PLA mats. The results also showed higher relative FDA values with respect to colony counting, which could be interpreted as a result of the existence of viable but non-culturable microorganisms associated to the inclusion of cobalt into fibers. Cobalt-based MOF included in electrospun mats provide antibacterial activity similar to that of the target nanomaterials and other fiber-based materials with antimicrobial functionality.

423 **Inactivation of antibiotic resistant bacteria in urban wastewater by solar advanced oxidation processes**

A. Frennet, University of Salerno / Civil Engineering; G. Ferro, Civil Engineering; M. Castro Alferez, M. Polo-López, Plataforma Solar de Almería-CIEMAT; P. Fernández-Biñaze, Plataforma Solar de Almería-CIEMAT; L. Rizzo, University of Salerno / Civil Engineering

In the present work solar disinfection and solar driven advanced oxidation processes (AOPs) were evaluated in the inactivation of indigenous antibiotic resistant bacteria (ARB) in real urban wastewater. H₂O₂/Sunlight, TiO₂/Sunlight, H₂O₂/TiO₂/Sunlight and natural photo-Fenton (wastewater pH unchanged) were the AOPs investigated. A multidrug (namely ampicillin, ciprofloxacin and tetracycline) resistant E. coli strain isolated from the effluent of the biological process of an urban wastewater treatment plant (UWTP) was the target ARB. The best disinfection efficiency was observed with TiO₂/Sunlight and H₂O₂/TiO₂/Sunlight, with an inactivation of the E. coli strain under the detection limit (2 CFU mL⁻¹) achieved for 3-5 KJ L⁻¹, depending on H₂O₂/TiO₂ ratio. A good performance was observed with H₂O₂/Sunlight process was observed with inactivation of E.coli achieved for 8 KJ L⁻¹. While with the Fe₂⁺/H₂O₂, at natural pH, the best performance of inactivation was reached with 15 KJ L⁻¹. Photo-Fenton was also effective in determining whether these new materials are significantly different than their natural counterparts. Similar processes have been studied for decades for naturally occurring nanoparticulate (1-100 nm) and colloidal (1-1000 nm) substances. The knowledge gained from these investigations is nowhere near sufficiently complete to create a detailed model of the behavior and fate of ENMs in the environment, but is a valuable starting point for the risk assessment of these novel materials.

424 **A decision tree model to determine the difference between engineered and natural materials**

S. Wagner, University of Vienna / Department of Environmental Geoscience; A. Gondikas, E. Neubauer, University of Vienna / Environmental Geosciences; T. Hofmann, University of Vienna / Department of Environmental Geosciences; F. Völler, University of Vienna / Department of Environmental Geosciences

There is no doubt that the development of nanotechnology leads to the emission of ENMs into the environment. Due to the measuring principle in both test systems (AM Viability and P. putida), with a parallel decrease in viable microorganisms, the effect was higher for *P. putida* that *S. aureus*, an effect that could be associated with the different way of transporting cobalt in gram-negative and gram-positive bacteria. The comparison between the amount of viable cells (FDA staining) and the number of CFU for cultures of *P. putida* and *S. aureus* showed a higher sensitivity of *P. putida* to the inclusion of cobalt in fibers with a reduction in the number of viable cells reaching 40% with respect to neat PLA mats. The results also showed higher relative FDA values with respect to colony counting, which could be interpreted as a result of the existence of viable but non-culturable microorganisms associated to the inclusion of cobalt into fibers. Cobalt-based MOF included in electrospun mats provide antibacterial activity similar to that of the target nanomaterials and other fiber-based materials with antimicrobial functionality.

425 **Repeated exposure - Effects of two nanomaterials on soil microorganisms**

K. Schlic, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; L. Beule, Fraunhohe IME; K. Hund-Rinke, Fraunhofer IME

Nanomaterials (NM) enter the environment by different pathways. A repeated exposure of NMs by sludge by multiple applications onto soil over years would be a realistic scenario. The aim of our study was to investigate the effects of CuO-NMs (Plasmachem GmbH) and AgNPs on soil microorganisms with the main focus on a repeated exposure scenario. At the single application 1.67 and 5.0 mg/kg soil for AgNM and 333 and 1000 mg/kg for CuO-NM were applied at test start. For the repeated exposure we used 0.56 and 1.67 mg/kg for AgNM and 111 and 333 mg/kg for CuO-NM. Here, the NMs were applied at day 0/28/56. Three test systems were used to assess the effect on the soil microorganisms: ammonium oxidation (ISO Guideline 15685), enzymatic activity patterns (ISO 22939) and MicroResp approach. Ammonium oxidizing bacteria were sensitive towards AgNM after 28 and 56 days of exposure independent of the application, whereas CuO-NM resulted only at high concentrations in an inhibition. For AgNM we found that, mainly the N- transformation was affected, observing the enzymatic activity patterns. Additionally, the C- and P-transformation was slightly affected at both the highest test concentration with single application and repeated exposure after 56 days. Applying the MicroResp approach, a more intense inhibition of the substrate induced respiration was observed. After 56 days we found that all transformations (C, N, P) were significantly inhibited at both the highest test concentration with single application and repeated exposure. Comparable results were observed for CuO-NM with a higher sensitivity of the N- and P- transformations compared to the C-transformations after 28 days. Currently, a repeated exposure resulting finally in the same test concentrations as a single application does not provide further information. The results of the study reveal that a low solubility and a consequent low ion release lead to a lower toxicity. The test duration is an important factor and should be extended to observe a slow long-term ion release. Tests for functional diversity reveal that using comparable carbon sources the test systems have a different sensitivity. Due to the measuring principle in both test systems different enzymes (exo-lendohexozymes) are addressed. The two test systems together provide a tool representing an early warning system for possible damages to soil microorganisms. Acknowledgement - The study was funded by the EU FP 7 MARINA and SUN.

426 **Establishment of AgNPs toxicity profiles and uptake routes in earthworms (E. fetida) combining Standard Toxicity Tests (OECD-207, 222) and coelomocyte biomarkers**

N. García, UPVEHU / Department of Zoology and Animal Cell Biology; M. Gandariasbeitia, E. Urionabarrenetxea, University of the Basque Country UPV-EHU; A. Izar Loibide, Aarhus University (AU) / Department of Bioscience; M. Soto, University of Basque Country / Zoology and Animal Cell Biology Research Centre for Experimental Marine Biology and Biotechnology PEU/VEHU

Silver nanoparticles (AgNPs) are extensively used in consumer products mainly due to their antimicrobial properties. AgNPs enter the terrestrial environment, though the land disposal of sewage sludge (wastewater treatment plant), or incineration and posterior deposition, and could modify the soil community structure and functions. However, tested AgNPs do not enter soils (either dissolved in pore water or coupled to soil organic matter) and their effects on soil organisms. Earthworms have been widely used for soil health assessment due to their pivotal role in the soil and their quick and measurable responses after exposures to pollutants. Soil health can be assessed measuring these responses (biomarkers) in their immune cells (coelomocytes) and with the aid of standardized toxicity tests. This work introduces the study of the in vivo toxicity profile of AgNPs combining Standard Toxicity Tests (OECD-207 and 222) with cell-level biomarkers (Neutral Red Uptake -NRU- and Calcein-AM Viability assays), and (b) to determine the uptake routes (dissolved, ingested) of AgNPs for a correct assessment of soil toxicity using *Eisenia fetida* earthworms. Earthworms were exposed to a range of concentrations of PVP-PEI coated AgNPs. Then Earthworm Acute Toxicity Test (OECD-207), including a Proper Contact toxicity test and Artificial Soil tests at short (3 d) and medium (14 d) terms, and the Earthworm Reproduction Test (OECD-222) were applied. The viability of coelomocytes was assessed by NRU and Calcein-AM tests. Automaticellography and Aelian Blue were used to address Ag distribution and morphological alterations in
the digestive tract and in the epidermis. Paper Contact test revealed a LC50 of 346.5 ppm Ag-NP and Artificial Soil test of 144.2 mg Ag-NP/kg. Histological and histochemical analyses proved that the primary uptake of AgNPs was via soil ingestion. A decrease in the number of viable cells occurred after 3 d of exposure to 50 mg Ag-NP/kg and after 14 d to 5 mg Ag-NP/kg. Reproduction was severely impaired at high Ag-NP doses. In conclusion, the combination of Standard Toxicity Tests and consecutive tests was useful to determine AgNP's uptake thresholds and to determine their uptake route by earthworms. This approach can be used for assessing the potential risks of AgNPs in soils. Acknowledgements: Basque Government (Grant to Cons. Res. Groups; IT810-13), Univ. Basque Country (UR1 11/37) and Spanish MINECO (Nanosilicovem Project).

427 Plants matter - Barriers, pathways and processes for uptake, translocation, and accumulation of nanomaterials in plants F. Schewab, Duke University / Civil Environmental Engineering CEINT; G. Zhai, M. Kern, The University of Iowa / Civil Environmental Engineering; A. Turner, Duke University; J.L. Schmoot, The University of Iowa / Civil Environmental Engineering; M. Wiesner, Duke University. Uptake, transport and toxicity of engineered nanomaterials (ENMs) into plant cells are complex processes that are currently still not well understood. Parts of this problem are the multifaceted plant anatomy, and analytical challenges to visualize and quantify ENMs in plants. Especially higher plants are the base of many food webs and paramount for human nutrition. To assess the toxicity, the exposure, and eventual uptake of ENMs, the focus needs to be on the uptake, transport and processes of ENMs in plants is needed. Numerous recent studies have documented ENM uptake into plants either by dissolution of the ENMs, or by uptake of particulate matter through the apoplastic. Still, the physiological features of plants favoring ENM uptake into the plants remain unknown, making it impossible to identify the most ENM-affected plant organ or group. [1] We report here on our review on the uptake, translocation, and accumulation of ENMs in plants, taking into account plant physiology and the physico-chemical properties of all relevant uptake barriers of higher and lower plants. Further, for the first time, based on the knowledge gained from plant uptake studies over the last ten years, an uptake model for ENMs will be presented and shown to be valid for studying uptake experiments performed under environmentally relevant conditions. Finally, the most important knowledge gaps will be analyzed. As a whole, the findings of the approximately 200 original studies performed during the last ten years on ENM translocation in plants show that the plant cell wall, long considered as an almost impassable barrier for ENMs, is much more permeable than previously assumed. The lack of observed toxicity may be due to a lack of specific toxicity of ENMs, but by the presence of detoxification reactions of the plants that effectively cope with ENMs. The next steps towards producing a realistic risk assessment of nanomaterials in plants are to measure ENM uptake rates, the size exclusion limit of the apoplasm, and to unravel plant physiological features favoring uptake. [1] Dietz KJ, Herth S. 2011. Plant nanotoxicology. Trends in Plant Science 16:582-589. [2] Schewab F. Zhai G. Kern M. Turner A. Schmoot J.L. – Environ., Life Sciences; Wiesner M. – Duke University. Uptake, Translocation, and Accumulation of Nanomaterials in Plants—Critical Review. Submitted to Nanotoxicology.

428 Quantity and quality of natural organic matter trigger the ecotoxicity of titanium dioxide nanoparticles to Daphnia magna F. Seitz, Inst. for Environmental Sciences / Institute for Environmental Sciences; R.R. Rosenfeldt, University of Koblenz-Landau, Institute for Environmental Sciences / Institute for Environmental Sciences; M. Müller, R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment. The heavy production and use of engineered nanoparticles likely leads to their unintentional release into aquatic ecosystems. There nanoparticles underlie a distinct fate, which is amongst others determined by the amount and properties of natural organic matter (NOM) ultimately modifying the nanoparticles’ ecotoxicological interactions. However, little is known about the underlying mechanisms, particularly which NOM properties (=quality) trigger such shifts. Therefore, the present study targeted the question how NOM quantity and quality alter the ecotoxicological potential of titanium dioxide nanoparticles (nTiO2) towards the water flea Daphnia magna. For this reason two nTiO2-products (A-100 and P25, ~100 nm), were investigated in combination with four NOMs of different quality, including four leachates of forest, agricultural and medium composition were also investigated. Preliminary results suggest that a) an increase in NOM quantity decreased toxicity by a factor >5 at higher NOM concentrations when compared to an exposure scenario without NOM. However, interactions of nTiO2 and NOM under various conditions such as further environmental and/or chemical stressors need to be understood to reliably predict the environmental risks of such nanoparticles.

429 The effect of silver nanoparticles on a freshwater alga (Pseudokirchneriella subcapitata) under three different design tests J. Curry, Heriot Watt University / Life Sciences; J. Kinross, Heriot-Watt University / School of Life Sciences; H. Johnston, Heriot Watt University / Life Sciences; T.F. Ferrarides, Heriot-Watt University / School of Life Sciences. Pseudokirchneriella subcapitata is a freshwater alga frequently used in standard toxicity tests; a primary producer with high ecological relevance. Project work done to date has focussed on silver nanoparticle toxicity, due to its frequent occurrence in consumer products. JRCNM03002a (formerly NM300K) was used as a reference; an aqueous dispersion of silver nanoparticles of a modal size of 15 nm, provided by the European Commission Joint Research Centre. Silver uptake was used to compare the effects of silver ions to their nanoparticulate form. Acute hazard studies allowed the derivation of EC50 values based on growth inhibition (OECD 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test). These were compared across 3 experimental designs – flasks, 24 well plates, and 96 well plates. Humic acid, pH and medium composition were also investigated. Preliminary results suggest sensitivity is dependent on media choice, but varies depending on the experimental approach. Growth inhibition tests have shown higher toxicity in a non-standard medium, compared to standard OECD medium. Growth inhibition decreased with exposure time (up to 72 hours) in all tests, for both ionic and nanoparticulate silver. This may be due to loss of toxicant to the test system, or the onset of tolerance in P. subcapitata, and requires further investigation. Standard toxicity tests offer a degree of flexibility in terms of test design, media, or target species. These results highlight the need for consideration of standard test deviations when comparing EC50s between laboratories. Small volumes and high replication may offer a robust approach of screening many toxicants in parallel, but care must be taken when comparing results between different methods. As this work has been carried out with silver nanomaterials, consideration must be made when extrapolating these results to other nanomaterials. Future work will investigate non-standard methods, including short term photo-inhibition tests and sub-betical bioassays. Cellular stains employed in mammalian toxicology will be investigated for their applicability to P. subcapitata. Such methods may be of practical utility to regulators due to higher replication and ease of sampling, compared to existing standard tests. Funding for this project was provided by the European Commission FP7 project MARINA (Managing the Risks of Nanomaterials), which seeks to develop and validate risk management methods for nanomaterials.

Prospective Life Cycle Thinking approaches for the definition and implementation of sustainability strategies in industry and policy making (I)

430 Product Environmental Footprint pilot phase - will it lead us to a harmonized life cycle based approach usable for comparative assessment? V.K. Bach, Technische Universität Berlin; A. Lehmann, Technische Universität Berlin / Department of Environmental Engineering Chair of Sustainable Engineering; M. Finkbeiner, DaimlerChrysler AG / Chair of Sustainable Engineering. The Product Environmental Footprint methodology (PEF) published in 2013 by the European Commission aims at harmonising existing methods, while decreasing the flexibility provided by ISO standards regarding methodological choices. Currently the method is tested for 25 pilots, which develop PEF specific product category rules (PCRs). These rules have to be valid for studies of up to 5 years, depending on the tested products. These data suggest a rather low ecotoxicological risk of nTiO2 in natural aquatic environments as its ecotoxicity is reduced in the presence of any NOM tested here. However, interactions of nTiO2 and NOM under various conditions such as further environmental and/or chemical stressors need to be understood to reliably predict the environmental risks of such nanoparticles.
well.

431 Global Principles and Practices for Hotspots Analysis
M. Barthel, WRAP / Food Sustainability; C. Harman, I.A. Faya, PE INTERNATIONAL, Inc.; P. Strothmann, UNEP/SETAC Life Cycle Initiative
A growing number of different analytical disciplines are using a method called ‘hotspots analysis’. It is being used to filter and distill often large volumes of information to identify and prioritise hotspots for further investigation or action by industry, governments and other stakeholders. Hotspots analysis is increasingly being used to address significant sustainability challenges, like unsustainable levels of resource use and waste; water scarcity and improved river catchment management; product sustainability and the setting of sectoral sustainability strategies; by helping to provide focus in an era of information overload. Currently no common global approach to hotspots analysis exists; nor has there been any effort to bring together or share best practice amongst those product sustainability initiatives currently involved in hotspots analysis. Neither do any accepted principles or guidance exist on how to translate and apply the results of this analysis into meaningful sustainability information for use by industry, governments and other stakeholders. To address these gaps, this research project aims to: Develop a common understanding of and map hotspots analysis approaches currently in use around the world, their application at different scales or levels of detail (e.g. how they can be applied at a national, sector and product category-level); and to share the learning and experiences derived from these existing approaches; Develop, agree and pilot a commonly-agreed methodological framework and global guidance for ‘hotspots’ analysis based on identified principles and best practices, as a means of accelerating and prioritising international action on key sustainability issues; To develop and agree a guidance document on the consistent and appropriate use of the sustainability information derived from hotspots analysis; Evaluate and, if possible, implement a range of options to bring together the findings from hotspots studies from different geographical regions and create a shared knowledge base that is able to provide a richer, global picture of sustainability hotspots in the economy and society; and the range of options or solutions available to address them. The presentation will provide an overview of the results of the mapping study that has recently been conducted. It will also provide initial insights into how a methodological framework could look like, drawing on the results of the mapping study.

432 LCA deployment in legislation - description of policy options for the example of CO2 legislation in automotive industry
A. Lehmann, Technische Universitaet Berlin / Department of Environmental Engineering Chair of Sustainable Engineering; M. Finkbeiner, DaimlerChrysler AG / Chair of Sustainable Engineering
Life Cycle (LCA) Thinking is on the political agenda and many industries are actively developing LCA approaches since many years. As, we think that it is in substance “right” to base environmental legislation on LCA, we started to explore and develop policy relevant scenarios. Integrating Life Cycle Assessment (LCA) into legislation. This research process included identification and prioritisation of various policy options, analysis of technical requirements, SWOT/RACER analysis and the development of scenarios for policy implementation. It was found that theoretically a broad range of options for implementing LCA into policies exists (e.g. mandatory or voluntary options, process- or performance based options) and that practically some of them are already implemented in European legislation. For four selected policy options, a deeper analysis was conducted; technical requirements (e.g. methodology, models, data) as well as strengths, weaknesses or acceptance from different stakeholder perspectives were described and implementation scenarios were developed. It was found, that some technical requirements are the same for both voluntary and mandatory policies and that sometimes, voluntary policy has stricter requirements. Moreover, it was shown that generally highest relevance regarding CO2 reduction, but also highest efforts for implementation seem to be related to the mandatory-performance-direct option. Moreover, it was shown, that robustness and credibility can principally be guaranteed by all policy options and that acceptance strongly depends on the perspective of the stakeholder groups. Finally, from the studies, recommendations policy makers (e.g. EC) conducted in the EU and beyond (in Japan, the US and China) complemented these results. In the first phase of the study promising policy options were identified without having indicated a clear analytical, scientific overall preference for one single policy option. It was shown that technical implementation strongly depends on the implementation level and that solutions for most technical requirements are already available, but that a consensus on proper setting of these requirements is missing. The research process will be continued, including a broader stakeholder dialogue in Europe, the US, Japan and China to communicate and refine the policy options as well as to specify possible implementation pathways for LCA deployment in legislation.

433 Economic value as a functional unit for environmental labelling
H.M. van der Werf, INRA, UMR SAS / Environnement et Agronomie; T. SALOU, INRA, UMR SAS / Environment and Agronomy
Sustainable development has become a major component of French public action. A law was passed on environmental labelling of products, to be based on a product’s life cycle. To implement this law a trial has been conducted, involving 168 companies. The French parliament produced a report, based on this trial, on the interest of environmental labelling. Regarding food, the report criticizes the functional units (FU) chosen: 100 g, 100 ml, as they do not reflect the functions of food. These FU will favour high-input intensive systems and result in higher impacts for products from input and organic systems aiming to produce quality products. This paper is a contribution to the debate on environmental labelling as a strategy to promote sustainability. We present our thoughts and a proposition on the most appropriate FU for environmental labelling of food and other consumer products. In order to illustrate our proposition we present a case study that explores the effect of the choice of FU on the ranking of agricultural products from production systems of conventional (cereal production versus organic agriculture). When using the FU live weight, organic animals had larger impact values than conventional animals. Per ha of land, organic animals had smaller impacts than conventional animals. Organic agriculture thus constitutes a less impacting mode of land use. When the FU economic value is used, organic animals have similar or lower values for eutrophication, lower values for climate change and higher or similar values for land occupation. The choice of FU is crucial. The FU economic value is attractive, as it considers product quality through the product’s price. The price difference between organic and conventional animals reflects a willingness of consumers to pay more for a product that is perceived to be of better quality. There often is a tradeoff between quality and quality. A mass-based FU tends to favour products from systems that focus on quantity rather than quality. An economic-based FU avoids this. An economic-based FU is well suited for environmental labelling of any type of consumer product, it allows the integration of positive rebound effects: consumers that purchase more expensive quality products will have less income available for other consumption. The views expressed here are those of the authors and should not be attributed to INRA.

434 GREENING BOOKS Sustainability strategy for the Publishing Sector
M. Escamilla, LEITAT / Sustainability Unit; C. Hidalgo, G. Ferrer, Leitat Technological Center / RD Safety - Sustainability Division; Panayella, El Tinter
GREENING BOOKS is an innovative strategy for the Publishing industry comprising scientific work, online tools and guidelines for industry and stakeholders involvement to tackle the environmental aspects of the sector throughout EcoPublishing. EcoPublishing is an innovative form of Apublishing, incorporating environmental criteria in the publication’s supply chain processes that optimize the negative environmental impacts that all phases of the production process (including production, distribution and end-of-use). Based on LCA methodology and results, an online simplified tool BookDAP was developed. It easily calculates the environmental producing quality products. More generally, an economic-value-based FU is very well suited for environmental labelling of any type of consumer product, it allows the integration of positive rebound effects: consumers that purchase more expensive quality products will have less income available for other consumption. The views expressed here are those of the authors and should not be attributed to INRA.
as numerous systems for describing and certifying the sustainability of buildings. Primarily, these efforts were focused on reducing the negative environmental impacts of the operation of buildings. But as the building efficiency increases, embodied impacts associated with the production, maintenance, and eventual end of life of buildings become more significant. This is a good reason for the key actors in the construction and property industry to intensify the discussion on this topic. However, different stakeholders have different decision making situations and objectives, as well as their technical knowledge vary widely. This paper deals with the question of how embodied impacts assessment can be integrated into the decision making of the main actors in order to improve sustainability in the construction industry. The focus is on the indicators “embodied energy” and “embodied greenhouse-gas emissions”, and considering that they represent a part of Life Cycle Assessment (LCA), they can provide a first step into the subject of embodied impacts especially for those actors without extensive LCA experience.

The analysis presented in the paper is partly built on the preliminary results of the ongoing project IEA Annex 57 “Evaluation of Embodied Energy & Carbon Dioxide Emissions for Building Construction” where the authors actively participate. More specifically, the paper discusses the most important questions and challenges in embodied energy and greenhouse-gas emissions considerations in decision making per selected actor group. A framework for the best possible use of existing information by these selected actor groups along the different stages of the building project is proposed to assist them in making informed decisions. Finally, the framework is organised in a matrix showing which decision making situations are typically related to each specific role of the selected actor groups, how these are influenced by this new requirement and what are the very specific data that can be fed into the different important decisions taken along the building project life cycle.

**Fate, Effects and Risk Assessment of Metals: Regulatory Perspective**

436 Assessing organism recovery after multiple pulse exposures to the trace metal magnesium, using Hydra viridissima (Green hydra)

A. Prouse, Ecotoxicology Research Group School of Applied Sciences; A.C. Hogan, RAE Environmental Consultants; N. Miles, Environment and Heritage Division Government Department of the Environment; A.J. Harford, Dept. of the Environment / ERISS Environmental Research Institute of the Supervising Scientist Division; R.A. van Dam, ERISS / Australian Government Department of the Environment; D. Nuget, RMIT University / School of Applied Sciences

Water quality guidelines for trace metals are usually based on results from organisms subject to continuous exposure to the metal. In mine releases however, aquatic organisms are often be subject to pulse exposure to metals. The time taken for organisms to recover from a pulsed toxicant exposure is an important consideration necessary when applying water quality guidelines (WQGs) to intermittent events in the environment. Organisms may appear to have recovered using standard toxicity testing methods but could carry residual toxicant or damage that may make them more sensitive to subsequent pulses. Such cumulative effects may render guidelines under-protective. The current study evaluated recovery of a sensitive freshwater cnidarian, Hydra viridissima following single, double and multiple pulse exposure to the trace element magnesium. H. viridissima were exposed to 4-h magnesium pulses of 790 and 1100 mg/L separated by 2, 10, 18, 24, 48 and 72-h recovery periods. Twenty-four hour pulses of 570, 910 and 940 mg/L were separated by 24, 48 and 168-h recovery periods. Apparent recovery after a single pulse of 4-h or 24-h was considered to have occurred once the population growth rate of the pulsed treatments returned to that of the controls. Double pulse toxicity tests were conducted using a second 4-h or 24-h Mg pulse to match the first pulse, with inter-pulse periods of 24, 72, 168 and 240 h. All treatments showed similar or reduced sensitivity to the second pulse when compared with sensitivity to the single pulse, indicating full recovery occurred prior to a second pulse-exposure. Five variations of equivalent time-weighted average concentrations were used to compare sensitivity of organisms to various pulse scenarios. The sensitivity of hydra to the multiple pulses was significantly lower than the time-weighted average continuous exposure response in three of the four scenarios tested, indicating that the short recovery periods of these recovery periods. The results will be utilised with that of other species to inform the use of a site-specific, duration-based WQG for Mg and provides an example of the use of empirical data in the regulation of toxicant pulses in the environment.

437 Setting Water Quality Objectives for the Rum Jungle Remediation Project

R. Smith, A. Markham, Hydrobiology Pty Ltd; T. Laurenton, Northern Territory Department of Mines and Energy

In early 2013 the Northern Territory Government Department of Mines and Energy completed a study of the environmental values downstream of the former Rum Jungle Mine site. The purpose of the study was to describe the receiving environment in terms of its key ecological and geomorphological characteristics, identify environmental values of importance to the stakeholders, particularly traditional Aboriginal owners (traditional owners), and set appropriate water quality objectives for rehabilitation of the mine based on those environmental values. The study was undertaken by a team of scientific experts with a range of relevant technical skills and a detailed knowledge of the area. The team undertook a careful review of historic data and scientific reports, conducted a field inspection and held consultation meetings with key stakeholders including traditional owner groups. From the information gained from this exercise, the relevant environmental values for each river reach from upstream of the mine area to the coast were identified, and draft water quality objectives developed from trigger values identified for each environmental value. Further stakeholder consultation resulted in the adoption of those water quality objectives for the remediation, pending further investigations to develop locally derived water quality objectives. This project will be described as an example of the application of the proposed water quality management framework that has been developed as part of the current revision of the ANZECC/ARMCANZ (2000) water quality guidelines.

438 Metal bioavailability: Identifying regulatory monitoring priorities

D. Leverett, A. Peters, Wca Environment Ltd.; R. Clarke, Sepa

The move towards setting Environmental Quality Standards for metals on the basis of "bioavailable metal" concentrations in Europe presents some challenges for regulators in assessing compliance against these standards. The change from standards which have typically been based on water hardness alone, or are not considered to be affected by water chemistry, means that the compliance situation for these metals may change. It cannot necessarily be assumed that areas where the potential risk due to metals has historically been perceived to be high will continue to be the greatest priority. An important problem which may environmental regulatory authorities throughout Europe are faced with is the lack of available monitoring data for the supporting parameters (principally pH, DOC, and calcium) which are required to perform metal bioavailability assessments. In order to maximise the number of locations for which metal bioavailability estimates could be made all of the available monitoring data obtained over a 20 year period was considered, thus average values for DOC and calcium were calculated for each site for all of the data available over the entire period. Metal bioavailability was calculated for the average bioavailability conditions at sites with reported metal exposures using chronic biotic ligand models for copper, manganese, nickel and zinc. Compliance against standards for cadmium and lead were assessed against the standards set under the Water Framework Directive, as a function of hardness and DOC concentrations respectively. The sensitivity of the receiving environment depends upon the local water chemistry, and the effect of water chemistry can differ for the different metals due to their differing interactions with both the biota and the water chemistry conditions. Initial indications are that overall the risk posed by metals in Scottish freshwaters is low, although there are a number of isolated sites with "bioavailable" concentrations of metals for which compliance against the standards is not possible. Sufficient water chemistry information to estimate metal bioavailability were not included in this assessment. The spatial distribution of the potential risks posed by each of the metals to the Scottish surface water environment will be presented, along with estimates of the cumulative risk posed by mixtures of metals in these situations.

439 Water quality assessment: the use of biotic ligand model-based software applications to address the bioavailability of metals

H. Ruedel, Fraunhofer UME - Institute for Molecular Biology and Applied Ecology / Environmental Monitoring

In contribution to the use of software applications based on biotic ligand models (BLM) for the evaluation of monitoring data of metals in the context of the European Water Framework Directive will be discussed. BLM-based applications have been developed to allow a user-friendly local evaluation of the bioavailability of metals. Therefore local quality standards (QS, protection goal: pelagic communities) are calculated as a function of water quality parameters. The original BLM software applications (Bio-Net (www.bio-net.ee) and PNEC pro (www.pnecrepro.com)) are available which allow both the evaluation for three metals (Cu, Ni and Zn). The dependencies of the calculated local QS on the parameters hardness, DOC concentration and pH are described and the results of the evaluation of representative scenarios for both applications compared. Based on the results of this study recommendations regarding the future use of BLM-based software applications for accounting the bioavailability of metals in the evaluation of water quality are derived.

440 Fate of tin in aqueous media: implications for the validity of ecotoxicity testing

I.A. Wilson, Institute for Molecular Biology and Applied Ecology

Soluble tin [Sn] salts undergo rapid precipitation in aqueous media which can cause problems when performing ecotoxicity tests on these substances. There is a need to meet regulatory requirements on the environmental risk assessment of Sn and Sn compounds, in particular reliable effects data from standard ecotoxicity testing.
Ecotoxicity tests in which effects are caused by physical factors by precipitated solid particles should not be used for either classification or risk assessment. It is essential to avoid test conditions which could result in the precipitation of material during the test. Experiments were performed with tin (IV) chloride at a range of environmentally relevant concentrations in OECD T-D test media at nominal pHs of 6, 8, and 8.5. The results demonstrated that the time to equilibrium in all tested materials was highly variable. The longest time for toxicity was observed at low spike concentrations and at high pH. The equilibrium concentration is controlled by the pH, the solubility of the possible precipitated solid phases, and the formation of negatively charged sites on the surface of the Sn precipitates may result in further binding of Sn cations. The data produced from the experiments was used to produce predictive Sn precipitation models which were used to predict the behavior of Sn in different environmental tests. These experimental and predictive toxicity testing failed the outline criteria due to the possibility that the test subjects were exposed to precipitating solutions during testing. For these historic tests it is not possible to establish the exposure concentration of tin to the aquatic organisms. How the results of any such testing might be used within a regulatory framework should be discussed with the relevant authorities prior to conducting any further tests.

441 Global assessment of manganese emissions from extraction and processing R. Marks, WCA Environment; A. Peters, WCA Environment Ltd.; D. McCough, A global environmental risk assessment has been conducted in order to assess the environmental risks due to manganese emissions from the manganese extraction and processing facilities. For this, a model of manganese transport, available, a qualitative assessment has been conducted to determine the potential environmental risk. For some sites the available data does not allow for a quantitative risk assessment, and for these sites a qualitative assessment has been carried out. This study provides a generic risk assessment of manganese production and use sites, in order to identify factors that affect risk to the environment. The principal sources producing or using manganese fall into three broad categories. These are mining sites, refining sites, and sites producing manganese emissions from refining sites and smelters with wastewater discharges is from the manganese to the surrounding environment from the sites. The principal sources of emissions from refining sites and smelters with wastewater discharges is from the treatment of emissions to air. This is typically carried out using wet scrubber air filters to collect the particulate materials. The effluent from this process is typically then treated on-site, with solid waste removed off-site, prior to discharge. Sites of this type may also hold relatively large stockpiles of raw materials on-site, and the interactions between these stockpiles may also potentially result in small environmental emissions of manganese. A generic risk assessment for direct emissions to surface waters has been conducted for several indicative tonnage bands. These emission calculations assume that wastewater discharges are treated on-site prior to release into surface water, where a limited degree of dilution may be available.

442 Risk assessment, risk management and mitigation for pesticides: from regulation to public perception I)

443 Effects of fungicides on decomposer communities and leaf decomposition in tropical regions D. Fernández, K. Voss, University Koblenz-Landau / Quantitative Landscape Ecology; J.P. Zubrod, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; M. Bischluch, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; R.B. Schuler, University Koblenz-Landau / Institute for Environmental Sciences. Large amounts of fungicides are applied globally and partly enter freshwater ecosystems. While effects of insecticides and herbicides on organic matter processing, which represents an important ecosystem process, have often been reported in the literature, only a few studies focused on the effects of fungicides. We present results from the first field study on the effects of fungicides on leaf decomposition and microbial and leaf-shredding macroinvertebrate communities. Moreover, compounds identified as ecotoxicologically relevant in the field were tested for effects in laboratory microcosms and in a stream mesocosm study, also considering potential interactions between nutrients and fungicides. The field study was conducted in 17 streams in a German vineyard area, where fungicides represent the most important pest control agents. Fungi and protozoa were monitored during 4 rainfall events using passive samplers. Fungicide toxicity ranged from ~4 to ~2 log units of toxic units. The structure of microbial and invertebrate communities changed along the gradient of fungicide toxicity. Changes in fungal community structure were also identified under laboratory conditions for fungicide mixtures from the field. Moreover, fungal biomass and richness decreased in the field in association with increasing toxicity. These changes might explain the reduction of the microbial OMD rate of up to 40% in polluted streams. By contrast, neither the invertebrate OMD rate nor the omnivour feeding rate correlated with fungicide toxicity. Our results suggest that fungicide exposure in the field can affect microbial communities and may reduce microbial OMD. However, further studies should test for causality of the observed associations and scrutinize effect mechanisms of fungicides on OMD.

444 Ecological risk assessment based on an ecosystem services approach for pesticides in tropical regions A.L. Sanchez, USP / São Carlos Engineering School; L. Mende, University of São Paulo; E.G. Espindola, University / Hydraulics and Sanitation

Ecological risk assessment studies are important to assess environmental changes that have been caused by various anthropogenic activities, such as leaching of contaminated areas, input of domestic and industrial sewage, runoff of agricultural areas and other impacts, which are responsible for physical, chemical and biological alterations. The study was conducted in a stream with known an impact from the other side of conservation: the goods and services that ecological systems can provide to human societies. These ecosystem services are the result of complex interactions between abiotic and biotic components and the evaluation structures are intended to include the role of human beings in these interactions. In this context, the focus of this research was evaluated the ecological risk assessment for pesticides and incorporating ecosystem services concepts for tropical regions. To attempt it, the risk scenario was architected based on recommended doses of the fungicide Pyrimethanil for agriculture cultures. For this simulation were used a gradients of concentrations, demonstrating multiple applications on crop situations through seed germination tests, elutriated tests coming from the plant tests with aquatic organisms and avoidance tests with soil organisms. The results obtained suggest that a study (based on chemical, ecotoxicological and toxicological measures), indicated that risks were higher in increasing the recommended dose. The ecotoxicological risks showed a toxicity trend with increasing the recommended dose for the different tests and tested organisms and for ecological parameters of the environmental risk analysis were used the changes and loss of the
ecosystem services concepts which are potentially affected by the fungicide. In addition were categorized the ecosystem services as provisioning for generation tests, regulating for elutriated tests and supporting for avoidance tests. This measurement showed more accuracy in the environmental impacts on the structure and functioning of natural ecosystems making it an important tool for risk management programs for the pesticides in the tropical regions. We concluded that the original ecological risk trends and potential projections of the studies for use in disturbances areas. Impacts were more relevant when included new analyzes factors such as the measurement of the effects of contaminants on the functions and ecosystem services in disturbances areas.

445 How effective are risk mitigation measures to protect surface water? A modelling approach

L. de Baan, O. Daniel, Agroscope / Institute for Plant Production Sciences IPS

To reduce unwanted side effects of pesticide use on surface water organisms, different mitigation measures exist. In Switzerland, a set of risk mitigation measures are in place. The aim of the present study was to evaluate the effect of different risk mitigation measures on the acute risk to aquatic organisms. We modelled how different pesticide usage scenarios in wheat affect the potential risk to a set of aquatic organisms. Five scenarios were evaluated: (1) no minimum distance to surface water, (2) minimum distance of 3 meters to surface water, (3) minimum distance of 6 meters to surface waters, (4) Product-specific buffer zones enacted in Switzerland by the end of 2013, (5) "Extender" label production: only applying herbicides and adhering to a minimum distance of 6 meters. SYNOPs was used to model the risk of a set of pesticide usage scenarios in wheat production in Switzerland, using pesticide survey data of totally 420 wheat fields from the Swiss agro-environmental monitoring program. Mandatory minimum distances of spray applications to surface water can largely contribute to minimize the risks of pesticide spray drift for aquatic environments, reducing the risk to about 1% (3m) and 2% (6m) respectively. The compliance with such measures is relatively easy to control compared to the compliance with product-specific buffer zones. For products where side-effects on water organisms are expected to be large, an additional, product-specific buffer zone to surface water bodies can reduce concentrations of these problematic products and the related ecototoxic effects by up to 100%. Label wheat production also significantly contributed to reduce risks and avoiding the usage of insecticides and fungicides could nearly bring the modelled risk to zero for water fleas, fish and midges. In summary, all risk mitigation measures showed positive effects and the combination of measures, e.g. scenarios 3 and 4 or scenario 5, is expected to be most effective to mitigate risks to acceptable levels. In this study, we modelled effects of risk mitigation measures for the extensively used case of wheat. If this product can be transferred to other cultures, especially to cultures with higher expected risk from spray drift such as orchards or vineyards, needs to be further evaluated.

446 The combined effect of diffuse pollution from agriculture and climate change on aquatic biota.

V. Silva, Universidade de Aveiro / Department of Environment and Planning and the Centre for Environmental and Marine Studies CESAM; C. Marques, University of Aveiro / Department of Biology; J. Keizer, Department of Environment Centre for Environmental and Marine Studies CESAM University of Aveiro Aveiro Portugal; F. Gónalves, University of Aveiro CESAM / Department of Biology; N. Alves, University of Aveiro / CESAM

Agriculture is already recognized as an important source of contaminants to the aquatic biota. Climate change poses an additional threat to these ecosystems, by increasing the water temperature and the concentration of contaminants, but the combined effects of stressors - diffuse pollution from agriculture and climate change - remains poorly understood. Therefore, through an experiment in microcosms (a more realistic scenario exposure), it was evaluated the combined effect of chemical contamination of water by copper sulfate, a fungicide commonly detected in water bodies adjacent to vineyard areas, in conjunction with a range of increases in water temperature on the response of freshwater species from different functional groups. Thus, the adverse effects on the growth of primary producers (alfalfa, Phragmites australis and macrophyte Lemna minor) and on the survival and feeding behavior of a shredder/decomposer species (Trichoptera Sclioptes sp.) were evaluated. The results showed that (i) copper-based fungicides, highly applied to control vine diseases such as downy or powdery mildew, can exert adverse effects on survival, growth and activity of aquatic organisms, reinforcing the problem of toxic effects of pesticides on non-target organisms and the need of more effective environmental management strategies. Furthermore, the results showed that (ii) the expected increases in water temperature by climate change will accelerate the metabolism of organisms at the same time that increases exposure and toxicity of contaminants. Provided the significant ecological roles of tested organisms (namely on the energy and nutrients flows), such results raise serious concerns about the global warming pressure exerted on water resources already impacted by diffuse pollution from agricultural sources.

Key-words: Experience in microcosm, diffuse pollution from agricultural sources, climate change, aquatic biota.

447 Developing ecological scenarios for the prospective risk assessment of pesticides

A. Rico, Wageningen University / Environmental Risk Assessment; T.C. Brock, Alterra / Environmental Risk Assessment; A. Focks, Alterra Wageningen University and Research Centre / Ecotoxicology Environmental Risk Assessment Team; P.J. van den Brink, Alterra Wageningen University

Mispredicted protection goals that underlie Ecological Risk Assessment (ERA) schemes for pesticides focus on the protection of populations of non-target organisms. Particularly in the case of terrestrial and aquatic non-target invertebrates and plants some pesticide effects may be allowed if population recovery is achieved within a given spatio-temporal frame. An important question for ecotoxicologists and risk evaluators is whether the combination of species and spatio-temporal frames currently used in ERA provide a realistic and conservative protection level for the exposed populations and communities under field conditions. We propose to approach this issue by systematically defining ecological scenarios that represent the impacted ecosystems and that can be linked to the already existing exposure scenarios to perform higher-tier evaluations e.g. using ecological models. In this study, we present and discuss a methodological approach for the formulation and implementation of ecological scenarios in the prospective risk assessment of pesticides, that can also be extrapolated to other chemicals. The proposed approach combines (bio)monitoring data, available toxicity data and relevant biological trait information to identify potentially vulnerable taxa. Following this approach, ecological scenarios will include: (1) a series of abiotic parameters (e.g. physico-chemical characteristics, descriptors of (agro)ecosystem management and landscape configuration), (2) a selection of vulnerable populations and their ecological interactions, (3) and a spatio-temporal frame that is relevant for the selected vulnerable populations. We propose the development of generic ecological scenarios for different landscape units (e.g. ponds, streams, ditches) in EU, and envisage three main areas in which ecological scenarios can be effectively used to support and improve ERA: (1) the identification of conflicts of interest between different protection goals, (2) the evaluation and design of laboratory and semi-field toxicity studies, and (3) the evaluation and motivation of the biotic and abiotic components included in ecotox(eco)logical modelling. In this presentation, we will provide practical examples on how ecological scenarios can aid to these three tasks by describing a case-study for Dutch agricultural ditches.

Reduction strategies of micropollutants from large and small-scale sewage water facilities (I)

448 Decision support for stakeholders in a river catchment: how to act on emerging contaminants

A. Fischer, Faculty of Civil Engineering and Geosciences; A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health; J. van der Hoek, Water Management

Emerging substances (ES) in surface water increasingly give concern to the public, regulators and users of this water. Water authorities and utilities struggle to define where and how to combat these substances in the water cycle. Different strategies are available; from preventing the compounds entering the water cycle to end-of-pipe solutions such as removal of the substances at drinking water treatment plants. In case of treatment, several technologies are available, which further complicates the selection of the most effective strategy. The EU Interreg project TAPES (Transnational Action Programme on Emerging Substances) aims to address this issue. Part of the project is to develop a decision support system (DSS) to facilitate local stakeholders with their task to decide on the most effective strategies to control ES within their segment of the water cycle. By interviews with various stakeholders, their main issues were identified: no overview of ES in water (sources, measures, treatment technology and location of measures) - how to locally define if a water body has problems with ES; what are the effects on health and the ecosystem? With these issues identified, a practical DSS framework has been outlined. A prototype is built based on this framework and will be shown. The final DSS, clustering existing knowledge, is giving the user information on the source, pathway, chemical characteristics and toxicity of individual emerging contaminants. Next, it provides information on the efficiency of various water treatment technologies in removing the specific substance. Finally, the DSS gives suggestions how to deal with relevant ES with regards to mitigation methods within the categories technical-, managerial- and social solutions The DSS prototype is used in two test cases. The first being a Belgian drinking water company that aims to expand their drinking water treatment with an extra step of advanced drinking water treatment. The second test case is a drinking water company where the DSS will be used to define what treatment steps are of interest to step specifically remove ES from wastewater effluent. The DSS will help actors in the water cycle for a better informed decision-making, and a more effective and uniform way of managing emerging substances.

449 Screening of micropollutants in wastewaters from on-site sewage treatment facilities and evaluation of current treatment techniques

M. Grox, Swedish University of Agricultural Sciences / Department of Aquatic
configuration, 14 campaigns have been planned in 2014 (8 already performed) to initiate to study at large scale a pilot (CarboPlus® plants is more and more considered by managers to reduce the emerging University / Leesu; s. zedek, LEESU laboratoire eaux environnement et systèmes powder and micro particles, and compared to the removal efficiencies observed in large scale STPs. Finally, a prioritization strategy based on removal efficiencies in OSSF facilities, concentration levels, frequency of detection and toxicity, persistence and bioaccumulation data, which were obtained from in silico tools, was applied to identify the most ecologically relevant micropollutants emitted from OSSFs.

450 Assessment of five different full-scale small wastewater treatment technologies for removing emerging contaminants V. Matamoros, Y. Rodriguez, IDAEA CSIC Nowadays, there is a lack of knowledge on the proportion that live in small communities (Daphnia magna). The concentration of ECs in the wastewater influents was higher than 10 µg/L for caffeine, paracetamol, methyl dihydrojasmonate and iproben, whereas most of the other compounds were found at concentrations below 1 µg/L. The removal efficiency ranged from negligible removal to more than 90% whereas most of the other compounds were found at concentrations below 1 µg/L. Therefore, approximately 80 micropollutants, belonging to different chemical classes, were successfully identified, such as per- and polyfluoralkyl substances (PFAS), pesticides, endocrine disrupting chemicals (EDCs), organophosphate flame retardants (OFRs), pharmaceuticals (PhACs) and personal care products (PCPs). Furthermore, the removal of identified micropollutants under small waste water treatment plants and soil bed treatments was assessed and compared to the removal efficiencies observed in large scale STPs. Finally, a prioritization strategy based on removal efficiencies in OSSF facilities, concentration levels, frequency of detection and toxicity, persistence and bioaccumulation data, which were obtained from in silico tools, was applied to identify the most ecologically relevant micropollutants emitted from OSSFs.

451 Removal of emerging micropollutants from wastewater discharges by powdered and micro-grain activated carbons - Case study of the CarboPlus® process R. Mailler, j. gasperi, LEESU / Leesa; Y. Coquet, SAUR; S. Deshayes, Paris-Est River / Leesa; s. zedek, LEESU laboratoire eaux environnement et systèmes urbains; A. Bule, Institut des Sciences Analytiques UMR TRACES Team / Service Central d'Analyse; E. Vulliet, CNRS; V. Eudes, Laboratoire Central de la Police Nationale; Paris-Est River / Leesa; Université Paris Est LEESU UMRMA UPEC ENPC AgroParisTech UPEMLV / Leesa; R. Moilleron, G. Chebbi, Paris-Est University / Leesa; V. Rocher, SIAAP The implementation of a tertiary treatment in conventional wastewater treatment plants is more and more considered by managers to reduce the emerging contaminant discharges into the aquatic environment. In this context, a cooperation between a sanitation service of Paris conurbation (SIAAP) and the Water Environment and Urban Systems laboratory (LEESU) has been initiated to study at large scale a pilot (CarboPlus® – SAUR/STEREAU) based on a high concentration fluidized bed of activated carbon (>100 kg/m³) with continuous fresh activated carbon injection. The objectives of this project are to: characterize the efficiency of the process for emerging pollutants and conventional wastewater quality parameters for powdered (PAC) and micro-grain (µGAC) activated carbons, and ii) characterize the parameters influencing the sorption mechanism (organic matter, operating parameters, activated carbon structure and properties, etc.). In addition to the 14 campaigns already performed in 2013 in PAC configuration, 14 campaigns have been planned in 2014 (8 already performed) to study the fate of 131 micropollutants, including 61 pharmaceuticals and hormones and 70 other emerging pollutants, in addition to conventional wastewater parameters. This presentation aims at displaying the pilot efficiency in µGAC configuration and comparing it to the PAC configuration. The use of µGAC instead of PAC has several operational and economical advantages, but this new type of carbon was never tested with wastewater. Based on the first 8 campaigns, the results tend to indicate that the µGAC is more efficient with µGAC than with PAC. In particular, ketoprofen, paracetamol, ibuprofen, ofloxacin, ciprofloxacin, trimethoprim, roxithromycin, atenolol, propranolol, carbamazepine, oxazepam, lorazepam and estrone have removals higher than 80% in average. In addition, first results on the other emerging micropollutants show high or very high removals for pesticides (60-95%), bisphenol A (60-90%), alkyphenols (60-95%), PFOS (75-95%) and X-ray contrast agents (50-70%). Moreover, the high solid retention time (60-90) days of the µGAC leads to a biological activity within the reactor, as displayed by NOx (65-99%) and NH₄ (20-70%) removals. However, the results are still partial at this stage of the project, another µGAC dose is currently tested on the pilot and complementary lab-scale tests are still performed to better understand the sorption mechanism.

452 Evaluating advanced biological wastewater treatment in a bioassay-based approach J. Völker, Aquatic Ecotoxicology; S. Castronovo, A. Wick, Federal Institute of Hydrology; T. Ternes, Bundesanstalt für Gewässerkunde; J. Ass, Process Engineering Institute Eawag; J. Oehlmann, Johann Wolfgang Goethe-Universität Frankfurt / Aquatic Ecotoxicology; M. Wagner, Goethe University Frankfurt / Aquatic Ecotoxicology During conventional activated sludge treatment of wastewater, micropollutants such as pharmaceuticals and biocides are often completely removed. This leads to a continuous discharge of a broad spectrum of emerging pollutants in the receiving water bodies and results in potentially adverse effects on aquatic ecosystems. One of the aims of the ERC project Athena is to explore the potentials and limits of various biological wastewater treatment processes for the removal of organic micropollutants and detoxification. Therefore, long-term experiments at pilot scale with 15 different set-ups were conducted directly at two medium-sized wastewater treatment plants (WWTP Koblenz, Germany and WWTP Duebendorf, Switzerland). The aim of the present study was to investigate the removal of toxicity by these processes using a broad battery of in vitro biosays. The pilot plant located at the WWTP Koblenz consisted of six 12 L sequence batch reactors fed with effluent of the primary clarifier. Based on previous results from micropollutant analysis, two promising treatments were selected for toxicity testing as pre- and post-treatment combined with conventional activated sludge treatment). For the in vitro analyses, we conducted four sampling campaigns in summer 2014. To investigate the removal of toxicity, SPE extracts were analysed in in vitro biossays covering a broad range of modes of actions, including endocrine and dioxin-like activity, cytotoxicity and genotoxicity. So far, the results indicated that the implementation of an anaerobic pre-treatment in addition to conventional biological treatment is a promising approach for a significant decrease of wastewater-borne toxicity. For example, an additional removal of estrogenic activity (Yeast Estrogen Screen) and bacterial cytotoxicity (Microtox) was observed. The pending results of further experiments will show whether this results can be confirmed. Sampling strategy, suitability of different in vitro endpoints and the results of an ongoing screening campaign will be presented and discussed. Financial support from the European Research Council (ERC) through the EU-project Athena is gratefully acknowledged.

453 Biodegradation kinetics and fate of Benzotriazoles and Hydroxy-benzotriazole in activated sludge and suspended biofilm systems A.A. Moaria, Aagean University Environment; A.S. Stasinakis, University of the Aagean / Department of Environment; H. Andersen, DTU Technical University of Denmark / Department of Environmental Engineering; Y. Pantazi, University of the Aagean / Department of Environment Benzotriazoles and benzotriazoles are two groups of compounds used in industries, particularly as corrosion inhibitors. They are found in wastewater concentrations of some ng L⁻¹ to several µg L⁻¹. They may cause serious damage to aquatic environment and organisms as well as creating problems in water reuse, when not eliminated efficiently. In order to examine and specify the elimination of 1H-benzotriazole (BTR), 5-Chloro-benzotriazine (CBTR), 5,6-dimethyl-1H-benzotriazole (XTR) and 2-hydroxy-benzotriazole (2HBHT) during biological treatment of emerging pollutants. They were found in wastewater after batch and continuous-flow conditions. Batch experiments were conducted in the presence and absence of easily degradable organic substances and biodegradation as well as sorption kinetics were calculated. Continuous flow experiments were conducted under different operational parameters to determine removal rates of target compounds. For this reason, two continuous-flow, lab-scale systems were set in the laboratory: i) an activated sludge unit consisted of aerobic reactor and settling tank and a Moving Bed Biofilm Reactor (MBBR) system consisted of two reactors in series filled with biocarriers. According to batch experiments, their sorption constants varied from 87 to 220 L Kg⁻¹, proving the general hypothesis that the compounds have a weak tendency to sorb onto organic matter. Biodegradation half
lives of target compounds varied from some hours to one or two days. The presence of easily degradable compounds seems to favor biodegradation of micropolutants, increasing their biodegradation constants. Removal rates in continuous flow experiments varied from lower than 50% (CBTR, XTR) to higher than 80% for 2OHHTH. To the best of our knowledge, this is the first study comparing the biodegradation ability of activated sludge and biomass attached to biocarriers.

### Alternative approaches for ecotoxicity assessments (II)

454 RTgill-W1 cell line assay for predicting fish acute toxicity: evaluation of a round-robin test

K. Schirmer, Eawag / Environmental Toxicology; M. Knobel, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology

We aim to establish alternatives to toxicity tests with fish for chemical risk assessment and product development. One recent development has been the rainbow trout (Oncorhyncus mykiss) embryonic (OME) bioassay, a free and cellular test to predict fish acute toxicity. Based on pre-defined selection criteria, 34 organic industrial chemicals were tested and compared to the acute toxicity reported for these same chemicals in the US EPA fathead minnow data base. The toxicity of the majority of test chemicals was within a 10-fold range from the line of unity of the cell line versus the acute fish lethality if true exposure concentrations were taken into account. Moreover, the outcome of the cell line assay compared very well to results obtained in the zebrafish embryo toxicity test, which has meanwhile been accepted as the OECD test guideline 226. These findings provided the impetus to start bringing the RTgill-W1 cell line assay as a potential animal replacement method to the same level of international acceptance. Thus, with the support of CEFIC-LRI and ECVAM-NCSRs, we initiated an international round-robin test to evaluate the transferability of the assay and intra- and inter-laboratory reproducibility. Seven laboratories provided data for this round-robin test either for testing chemicals in the cell line assay and/or confirmation of chemical concentrations in the testing plates at the start and end of the exposure period. These laboratories were, besides Eawag: Givaudan Schweiz AG (Switzerland); IRAS-Unichierto Research (The Netherlands); The Procter & Gamble Company (USA); NDV (Norway); RECETOX (Czech Republic); Vito-ABS team (Belgium). Chemical selection and quantitative analysis as well as the cell line assay followed previously described criteria and protocols. This RTgill-W1 cell line assay procedure was transferred in a straightforward manner to the participating laboratories. Further improvements to the original SOP were implemented based on the suggestions provided by the participants and, in fact, this round-robin test could provide a solid base to quantify the intra- and inter-laboratory variability and help to further establish this assay as an animal-free alternative to the fish acute toxicity test.

455 Effects of plastic and serum protein binding on cytotoxicity of catonic surfactants in vitro

N. Timmer, Utrecht University / Institute for Risk Assessment Sciences; F.A. Groothuis, Institute for Risk Assessment Sciences; E. Opsahl, Utrecht University / Institute for Risk Assessment Sciences; B. Nicol, Unilever RD Colworth / Safety and Environmental Assurance Centre SEAC; N.J. Kramer, Utrecht University / Institute for Risk Assessment Sciences; s. T. droge, Utrecht University / IRAS

In vitro toxicity assays are envisaged to play a fundamental role in the risk assessment of industrial chemicals. Unfortunately, binding of chemicals to well plate plastic and serum constituents like bovine serum albumin (BSA) may lead to an underestimation of the toxicity. Binding affinities of cationic surfactants to such matrices is poorly understood. Therefore, this study assessed the cytotoxicity of twelve catonic surfactants, varying in carbon chain length (6-18 carbons) and ionic head group, in a basal cytotoxicity assay with the RTgill-W1 cell line. Cells in 48 and 96 well microtiter plates were exposed for 24-48 hours in serum-free exposure medium or medium containing either BSA (4 g/L) or 10% fetal bovine serum (FBS). After exposure, chemical concentrations in exposure medium and cells were measured using LC-MS/MS. Based on the nominal concentration of chemical in BSA/FBS free exposure medium, the cytotoxicity of benzalkonium chlorides increased almost 1000 fold with increasing chain length (and thus with hydrophobicity) but, not after the alky chain contained more than 10 carbon atoms. Based on measured concentrations in BSA/FBS free exposure medium, however, the longer chained benzalkonium chlorides were significantly more toxic. Effect concentrations measured in BSA/FBS containing medium were up to a 60 fold higher. The findings indicate that the greater the chain length, the stronger the chemical binds to plastic and Serum constituents, the lower the available fraction of the chemical is for causing cytotoxicity in cells. Based on cell-associated concentrations, there was little difference in the cytotoxic potency of the test chemicals because it is independent of assay setup. Such dose is related to the chemicals modes of action (narcosis). Thus, using free and cell-associated concentrations allows for a more robust comparison of intrinsic cytotoxic potencies of surfactants in vitro.

456 Primary gill cell culture representing organ functional integrity: Uptake, efflux and the first evidence of xenobiotic metabolism

L.C. Stott, Kings College, London / Division of Diabetes and Nutritional Sciences; S. Schnelli, Division of Diabetes and Nutritional Sciences; C. Hogstrand, Kings College London / Division of Diabetes and Nutritional Sciences; S.F. Owen, AstraZeneca / Safety Health Environment; N.R. Bury, Kings College London / Division of Diabetes and Nutritional Sciences

Worldwide, large numbers of fish are used in toxicology testing to determine the environmental effects of compounds that may be released into the aquatic ecosystem. In the UK last year, this number exceeded 42,000 fish. To find alternative toxicological data for fish, we have initiated a round robin exercise where a RTgill-W1 cell line was exposed to a free and cellular test to predict fish acute toxicity. Based on pre-defined selection criteria, 34 organic industrial chemicals were tested and compared to the acute toxicity reported for these same chemicals in the US EPA fathead minnow data base. The toxicity of the majority of test chemicals was within a 10-fold range from the line of unity of the cell line versus the acute fish lethality if true exposure concentrations were taken into account. Moreover, the outcome of the cell line assay compared very well to results obtained in the zebrafish embryo toxicity test, which has meanwhile been accepted as the OECD test guideline 226. These findings provided the impetus to start bringing the RTgill-W1 cell line assay as a potential animal replacement method to the same level of international acceptance. Thus, with the support of CEFIC-LRI and ECVAM-NCSRs, we initiated an international round-robin test to evaluate the transferability of the assay and intra- and inter-laboratory reproducibility. Seven laboratories provided data for this round-robin test either for testing chemicals in the cell line assay and/or confirmation of chemical concentrations in the testing plates at the start and end of the exposure period. These laboratories were, besides Eawag: Givaudan Schweiz AG (Switzerland); IRAS-Unichierto Research (The Netherlands); The Procter & Gamble Company (USA); NDV (Norway); RECETOX (Czech Republic); Vito-ABS team (Belgium). Chemical selection and quantitative analysis as well as the cell line assay followed previously described criteria and protocols. This RTgill-W1 cell line assay procedure was transferred in a straightforward manner to the participating laboratories. Further improvements to the original SOP were implemented based on the suggestions provided by the participants and, in fact, this round-robin test could provide a solid base to quantify the intra- and inter-laboratory variability and help to further establish this assay as an animal-free alternative to the fish acute toxicity test.

457 Establishment of the first humpback whale cell lines and their application in chemical risk assessment

M. Griffith University / Southern Ocean Persistent Organico Organic Pollution Program; D. Whitworth, The University of Queensland / School of Veterinary Science; K. Schirmer, Eawag / Environmental Toxicology; S.M. Bengtson Nash, Griffith University / Southern Ocean Persistent Organico Organic Pollutants Program SPOOPP

Humpback whales have a high propensity for the accumulation of persistent organic pollutants (POPs) at high trophic levels and are of concern to the health risks they pose. Few efforts have focused on the toxicological sensitivity of humpback whales due to the challenges associated with working on wild populations of large whales. Practical tools for deriving species-specific data has been identified as a pressing research need. This paper reports the first successful derivation and characterization of humpback whale cell lines. Primary cells were isolated from the dermal connective tissue biopsies, cultured at 37°C and 5% CO2 in the standard mammalian medium DMEM/F12 supplemented with 10% fetal bovine serum (FBS). Of nine initial biopsies, two cell lines were established from two different animals and designated HuWa1 and HuWa2. The cells have a stable karyotype with 2n = 44, which is identical to other baleen species. Cells were verified as being fibroblasts based on their spindle-shaped morphology and positive immunoreaction to vimentin. Population doubling time was determined to be ~41 h and cells were successfully cryopreserved and thawed. To date, HuWa1 cells have been propagated 20 times. Cells proliferate at the tested temperatures, 30, 35 and 37°C, but show highest rate of proliferation at 37°C. Short-term exposure to para,para'-dichlorodiphenylchloroethene (p,p'-DDE), a priority compound accumulated in humpback whales, caused a 50% decrease in cell viability. The effective concentration which caused a 50% reduction in HuWa cell viability (EC50 value) was approximately six times greater than the EC50 value for the same chemical measured with human dermal fibroblasts. HuWa1 exposed to a natural p,p'-DDE-containing chemical mixture extracted from whale blubber showed distinctively higher sensitivity than to p,p'-DDE alone. Thus, we provide the first toxicological data for humpback whales and with establishment of the HuWa cell lines, a novel in vitro model for the study of the whales’ sensitivity and cellular response to chemicals and other environmental stressors.

458 The fish embryo toxicity test as an alternative to the larval growth and survival test: Test sensitivity and alternative endpoints in zebrasfish and fathead minnows

M.K. Sellin Jeffries, Texas Christian University / Department of Biology; A. Stultz, Miami University / Department of Zoology; A. Smith, Miami University; D.A. Stephens, K.S. Roush, Texas Christian University / Department of Biology; J.M.
<p>Freshwater planarians in ecotoxicology studies. This work aimed to present methodologies that can be easily performed in laboratory assays while advocating the suitability of using freshwater planarians as an alternative for animal testing in ecotoxicological research. Keywords: freshwater flatworms; ecotoxicology; behavioural endpoints; regeneration</p>
experiments can be individually tracked until so. Indeed, progressive decreases of both markers were clearly observed. They are promising indicators of snail’s health. Last, we investigated the effects of a case xenobiotic: Etoposide. This anti-cancer drug is used worldwide. Etoposide induced a sharp drop of hemocytes at therapeutic-like concentrations [immunosuppression], whereas the density was increased at environmental realistic concentrations. Such phenomenon has been reported elsewhere in L. stagnalis; underlying mechanisms are not elucidated yet. Hemocyte viability is gradually affected by increasing drug concentrations. Phagocytosis activity remained unaffected. Further studies will look at the relationship between modulation of immunocompetence due to xenobiotics and Darwinian fitness endpoints to counterbalance the sensitivity of immunomarkers in the context of ecotoxicology.

463 Relationship between diuron biotransformation assessed by UPLC MS-MS and its embryo- and genotoxic effects in the Japanese oyster

D. Behrens, Laboratory of Ecotoxicology; N. Tapie, ISM/LPTC - UMR5255 CNRS; F. Pardon, University of Bordeaux / UMR CNRS 5805 EPOC-LPTC; H. Bautista, University of Bordeaux / UMR EPOC Equipe LPTC; J. Rouel, IFREMER; T. Burgeot, IFREMER / BIOGEOCHIMIE ECOTOXICOLOGIE; F. Akcha, IFREMER / Laboratory of Ecotoxicology

The oyster Crassostrea gigas is France principal shellfish aquaculture species but its production has been declining since the late 2000s due to recurrent summer mortality events. Although these episodes are believed to be multifactorial, recent studies have shown that oyster development is sensitive to pollutants such as herbicides. However, the mechanisms by which herbicides can affect oyster physiology remain unclear, as well as their biological fate once in the organisms. In this study, we compared the embryotoxic and genotoxic effects of the herbicide diuron and its known metabolites: DCPMU, DCPU and 3,4-DCDA. A range of environmentally relevant concentrations (0.002-0.5 μg L⁻¹) was tested applying the embryonic or the larval bioassay for the embryotoxicity study and the comet assay for genotoxicity assessment. Co-exposures of the herbicides (diuron or metabolites at 0.5 μg L⁻¹) with a known antioxidant compound (ascorbic acid at 5-25 μM) were also realised to check ROS involvement in the toxicity. The ability of oyster to biotransform diuron was also investigated at different live stages (spom, ooyte, trophiophore larvae, adult). Micromosual incubations of subcellular fractions with diuron (1.17 and 11.7 μM L⁻¹) were performed and biotransformation products analysed by LC-MS-MS. Diuron significantly impaired embryo-larval development from 0.01 μg L⁻¹. Although in a lower extent, N-demethylated DCPMU and DCPU triggered similar significant alteration. Only the final degradation product 3,4-DCPMU and DCPU triggered similar significant alteration. Only the final treatment 3,4-DCDA did not show any embryotoxic effect. The embryotoxicity of diuron, DCPMU and DCPU were significantly reduced in the presence of the antioxidant scavenger, highlighting a role for ROS production in diuron toxicity. That is in accordance with the oxidative DNA damage observed during this study. Preliminary LC-MS/MS analysis showed that the different live stages have the potential to biotransform diuron into DCPMU and DCPU, although in different extent. ROS production could be a side-effect of biotransformation process.

464 Nuclear receptors in the Pacific oyster, Crassostrea gigas, and their expression during early development

S. Vosseler, University of Exeter / College of Life and Environmental Science; T.S. Goulburn, University of Exeter / Biosciences Department; B.P. Lyons; T. Bean, Centre for Environment Fisheries and Aquaculture Science / Environment and Animal Health

Molluscs represent the second largest phylum among the invertebrates and are characteristic features of aquatic and terrestrial habitats worldwide. They are under enormous risk from xenobiotic compounds in the environment through anthropogenic pollutants, but little is known of the molecular mechanisms underlying these effects. Nuclear receptors are a superfamiliy of transcription factors important in key biological, developmental and reproductive processes. Several of these receptors are ligand-activated and through their ability to bind endogenous and xenogenous ligands, are potentially vulnerable to xenobiotics. By using the Pacific oyster (Crassostrea gigas) genome and a highly diverse set of nuclear receptors showing 43 homolog members to most of the known Drosophila and human receptors. They include members of the NR0-NR5 subfamily, notably lacking any NR6 members. Homologues to known xenobiotic-vulnerable nuclear receptors in other invertebrates and vertebrates have been confirmed (e.g. retinoid and thyroid – like receptors). Conducted gene expression analysis of the C. gigas nuclear receptors during early developmental stages using quantitative real-time PCR indicates that many of these nuclear receptors are crucial during organogenesis and shell development. This data provides a deeper insight in nuclear receptor presence and function in mollusc species and suggests that any putative xenobiotic-receptor interaction could lead to serious effects on the oyster development. This will directly aid the development of ecotoxicological risk assessments and enhance protection of the environment.

Multistress in aquatic environments: the big picture (II)

465 Ecotoxicology - Effects of Micropollutants from Wastewater Treatment Plants on Stream Ecosystems: Ecotoxicological and Chemical Evaluations in 24 Swiss Rivers

C. Kuenli; S. Birrer, Swiss Centre for Applied Ecotoxicology Eawag-EFPL; F. Diederer, Eawag Swiss Federal Institute of Aquatic Science and Technology; B. Gossner, Centre for Environment Fisheries and Aquaculture Science / Eawag-EFPL; J. Mazemauer, Eawag Swiss Federal Institute of Aquatic Science and Technology; M. Reyes; S. Santiago, Sowul Santiago; A. Schifferli, Swiss Centre for Applied Ecotoxicology Eawag-EFPL; H. Singer, Eawag Aquatic Research; B. Spycher, Eawag Swiss Federal Institute of Aquatic Science and Technology; E. Vermeiren, Eawag / Dept of Environmental Toxicology; C. Stamm, Department of Environmental Chemistry, I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology

Micropollutants are organic and inorganic substances, which occur in very low concentrations in surface waters. Even at these low concentrations, some of them can elicit effects on aquatic organisms. The aim of the project Ecotoxicity is to evaluate the effects of micropollutants originating from wastewater treatment plant (WWTP) effluents on stream ecosystems. The project combines chemical, microbiological, ecotoxicological and ecological evaluations in order to enable a comprehensive understanding of the river ecosystem processes. This paper focuses on a part of the project: the ecotoxicological and chemical evaluations. Several measurement campaigns were conducted in 2013 and 2014. In 2013 a screening of 485 micropolutants as well as laboratory and field bioassays were performed. At selected sites, feeding activity of amphipods (Gammarus fossarum) as well as the reproduction of water fleas (Daphnia pulex) was measured. Additionally, effects on the photosynthesis and growth of single-celled green algae (Pseudokirchneriella subcapitata), estrogenic activity (Yeast Estrogen Screen), and neurotoxic effects (acetylcholinesterase inhibition assay) were evaluated in all samples. 2014 assessment was performed in all sites, as well as additional sites of 60 substances as well as an assessment of estrogenic activity, algal toxicity and neurotoxic effects. With regard to estrogenic activity and photosynthesis inhibition, the impact of the WWTP was obvious. 17β-Estradiol equivalent concentrations (E2EQ) as well as diuron equivalent concentrations (DEQ) were mostly higher downstream compared to upstream of the WWTP. Similarly, a decreased amphipod feeding activity was detected at one of four sampling sites. A significantly decreased reproduction of C. dubia was detected at one of four sites as well. The applied methods proved to be well suitable for a chemical and ecotoxicological assessment of river water quality. In general, the contamination with estrogenic substances can be considered relatively low. The EEQs never exceeded the effect level for chronic (E2EQ) for 17β-estradiol (0.4 ng/L). However, in a majority of the evaluated rivers, the DEQ exceeded the AA-EQQ for diuron (20 ng/L), partly already upstream of the WWTP. Future data evaluation will include a comparison with chemical and ecological data to complete the overall picture, and to draw further conclusions on the effects of micropollutants on stream ecosystems.

466 Data opportunities and challenges for assessing multiple stressors across scales in aquatic ecosystems

K.A. Dafforn, University of New South Wales / Ecology and Evolution Research Centre; E.L. Johnston, University of New South Wales / Ecology and Evolution Research Centre; A. Ferguson, NSW Office of Environment and Heritage; C. Humphrey, Environmental Research Institute of the Supervising Scientist; W. Monk, Environment Canada; S.J. Nichols, University of Canberra / Institute for Applied Ecology and MDBFutures Collaborative Research Network; S.L. Simpson, CSIRO Land and Water / Centre for Environmental Contaminants Research; M.G. Tulbure, University of New South Wales; D.J. Baird, Environment Australia

Globally, aquatic ecosystems are under threat from a complex array of stressors, which vary in intensity and spatial scale. Monitoring and assessment of both marine and freshwater ecosystems have historically focused on collection of information on habitat quality, in terms of descriptions of physical and chemical conditions, and the associated sociated observations (biological condition). The advent of high-performance computing systems, coupled with new earth observation platforms, has accelerated the adoption of remote sensing tools and geographical information systems (GIS) in ecosystem assessment. Their use in interpretation of scale-dependent ecological phenomena is an emerging area, and here we explore synergies with key areas of stress ecology. We introduce a conceptual framework that outlines a strategy for assessing multiple stressors across ecosystems using emerging sources of “big” data. We define big data as any collection of data sets so large and complex that it becomes difficult to process using traditional data processing applications. We critique a range of available big data types that could support models for multiple stressors and the assessment of their effects.

467 Effects of organic pollutants on oceanic photosynthetic function mediated by Prochlorococcus

M. Fernandez-Pinos, B. Pina, IDAEA-CSIC / Environmental Chemistry; m. vila-costas, M. Casado, Environmental Chemistry; S. Agusti, IMDEA-CSIC; J.
Effects and accumulation process of organic pollutants and metals in bacteria, phytoplankton and zooplankton

470 Evaluating relations between stressors and ecological endpoints at the regional scale
P.C. Van Metre, U. S. Geological Survey / Texas Water Science Center; M.P. Babut, Iresta / Water; M. Liess, UFZ Center for Environmental Research / Department of SystemEcotoxicology

Relations between environmental stressors in streams—contaminants, nutrients, streamflow, temperature, and habitat—and ecological communities are highly complex. Effects of stressors on biological conditions often are evaluated in local-scale field studies, where processes can be monitored, or in laboratory experiments, where conditions can be manipulated to identify the cause(s) of impairment. However, results of local-scale studies are not necessarily applicable to other locations or able to describe large-scale patterns, and it is often difficult to extrapolate from the laboratory to the field. At the opposite end of the spatial scale, from local-scale studies are regional-scale studies that include measurements of stressors and ecological conditions at large numbers of sites across ecologically-similar regions with the goal of empirically modeling stressor-condition relations on a regional basis. Such studies can potentially provide the statistical power necessary to evaluate the effects of multiple stressors on ecological communities, and develop models to predict ecological conditions at unmonitored sites. Regional assessment approaches of streams will be illustrated by using results from the U.S. Geological Survey Regional Stream Quality Assessment of 100 sites in the Midwestern United States, an assessment of pesticide effects on macroinvertebrate communities using 663 sites across Germany, and development of a stressor-based ecological model using 1,100 sites across France.

471 Accumulation and trophic transfer of POPs in plankton in the Gulf of Lion, Western Mediterranean
J.A. Tronczynski, IFREMER / RBE; C. Tixier, IFREMER; M. Harmelin-Vivien, F. Carlotti, MIO

The present study focuses on the accumulation and trophic transfer of persistent organic contaminants (PCBs, PBDEs, OCPs and PAHs) at the primary trophic levels (autotrophs and heterotrophs) in the Gulf of Lion (GoL), Western Mediterranean. The concentrations of selected POPs in plankton were generally higher in the eastern part of the GoL i.e. at the stations under direct influence of urban center of Marseille and of the Rhône River. The results show that levels of PCBs and PBDEs in plankton in the GoL are spatially influenced by the distance from contamination source and that marine plankton seems to be able to assimilate large concentrations of POPs compounds at the stations near contaminants source. The relationship between POP concentrations and size class of plankton and plankton δ13C signatures is compound, seasonally and spatially depended. However, the highest concentrations of POPs were determined in the lowest fraction 0.7 - 63 µm of plankton. This fraction show significantly different δ13C and δ15N signatures from other plankton fractions, indicating high contribution of bacteria/nano and picoplankton to organic carbon pool in these samples. The role of this fraction in contaminants transfer within planktonic communities is yet poorly documented. Furthermore, our results indicate that prey-predator contaminant transfer is difficult to clearly distinguish within planktonic food-webs. The higher zooplankton size classes are composed of organisms with different diets, including herbivores, carnivores and detritivores, prone in addition to adapt their diet to the quantity of available resources. Additionally, in field samples, size class composition and isotopic signatures do not necessarily reflect a prey-predator relationships, and complex ecology and ecosystem interactions within plankton communities. Thus, evidencing and quantifying contaminant biomagnification in plankton may be a difficult task, especially in the coastal environments. The full data and results of this study focused on the trophic transfer of organic contaminants in plankton will be further discussed and presented.

472 Role of the Accumulation in Plankton on the Cycling of Polycyclic Aromatic Hydrocarbons in the Global Tropical Oceans
B. González-García, Institute of Environmental Assessment and Water Research / Environmental Chemistry; M. Fernandez-Escolà, IDAEA-CSIC / Environmental Chemistry; C.J. Galbán, Sustainability Research Centre / Department of Ecology and Biodiversity; B. Jimenez, IQOOG-CSIC / Instrumental Analysis Environment; J. Dachs, IDAEA-CSIC / Environmental Chemistry

The presence of polycyclic aromatic hydrocarbons (PAHs) in the marine environment is a global concern due to their persistence and toxicity, as well as their potential for bioaccumulation and biomagnification in food webs. The study of the cycling of PAHs in the marine environment is crucial for understanding the ecological impacts of these compounds. The research presented in this session will focus on the role of plankton in the cycling of PAHs in the Global Tropical Oceans. The study will evaluate the accumulation of PAHs in plankton and its potential impact on the ecosystem. The research team will present their findings on the distribution and accumulation of PAHs in various species of plankton and the potential ecological effects of these compounds in the marine environment. The study will provide a comprehensive understanding of the cycling of PAHs in the Global Tropical Oceans and will contribute to the conservation and management of marine ecosystems.
Polycyclic Aromatic Hydrocarbons (PAHs) are organic pollutants generated during incomplete combustion of fossil fuels and organic matter. They cause carcinogenic and toxic effects in biota, and have been proved to be harmful for ecosystems. Moreover, PAHs are ubiquitous in the environment and show increasing levels in some regions due to growing anthropogenic sources. The global ocean has been pointed to hold a strong degradation capacity for these compounds, but it is still necessary to determine the entry and fate of PAHs in the ocean and how it is connected to the adsorption, biodegradation, and biotransformation processes occurring in the surface oceans. Atmospheric deposition is the main vector for the entrance of semivolatile organic compounds to the global oceans through two different inputs: dry deposition and diffusive air-water exchange. Dry deposition fluxes have been measured, and diffusive fluxes have been estimated from air and water concentrations, for all sub- and tropical oceans (35°S–40°S) during the Malaspina 2010 circumnavigation cruise in the Atlantic, Pacific and Indian oceans. Water and plankton concentrations of these compounds during the same sampling campaign were also measured. Low molecular weight PAHs, are prone to be diffused and degraded in the marine euphotic area, meanwhile high molecular PAHs (the more recalcitrant and toxic) enter into the ocean mainly attached to the aerosol and rapidly adsorb to plankton due to their lipophilicity. Therefore, a complex feedback is established between depositional fluxes and degradation/biodegradation processes and will be reviewed in this oral platform. Water-plankton equilibria and other physico-chemical processes in the water column (like PAHs “dilution effect” with high plankton biomasses) will be discussed as well. These results are the most extensive data set available for the global ocean and provide evidence of the interaction between physical and biological controls on PAH occurrence and cycling in oceanic regions.

473 Induced tolerance and altered microbial community composition after exposure to low concentrations of PAHs

E. L. Cockburn, Shipley / Marine Technology; I. Dahllof, University of Gothenburg / Department of Biological and Environmental Sciences; H. Landquist, Chalmers University of Technology; I. Hassellö, Chalmers University of Technology / Department of Shipping and Marine Technology

When biotic communities are exposed to non-lethal concentrations for long periods of time there is a possibility that they develop tolerance to the pollutant. In that case the negative effects might be hidden through adaptation to the polluted conditions, hence the communities appear healthy. Polycyclic aromatic hydrocarbons (PAHs) are worldwide pollutants that lead to lethal effects in high concentrations and sub-lethal effects in low concentrations. The tendency for microbial communities to develop tolerance to PAHs is not well studied. In this study the ability for marine microbes to develop tolerance to PAHs was examined in a 90-day experiment. Treatments with an increase in PAH levels of 1.6 (H) and 0.3 (L) times were compared to control sediment. Day 30, 60 and 90 the microbes were exposed to a new addition of PAHs in a short-term toxicity test to detect possible tolerance. Ability for nitrification and denitrification by the microbial community was used as endpoints. Molecular analysis of the microbial communities was performed to detect possible differences in proportions of nitrifying bacteria compared to total bacterial abundance between treatments and control. We here present novel data showing an induced tolerance in nitrifying microbial communities to PAHs after 60 and 90 days. EC10-values for inhibition of nitrification were significantly higher in L after 60 days and both treatments after 90 days compared to control. Furthermore, the developed tolerance led to less productive communities when unexposed. It was also shown that the presence of non-competitive aromatic oxidizing bacteria vs. total bacterial density between treatments and control, indicating that development of the tolerance had varying pathways in the two treatments. The results from this study have implications on field studies or environmental monitoring programs of long-term oil and PAH contaminated sites. The potential development of tolerance must be taken into account and tested for when evaluating marine microbial health in oil contaminated areas, as tolerant communities can be wrongly mistaken for healthy communities. In addition, changed ratios of nitrifying microbes can alter the capacity for ammonium oxidizing in benthic marine sediments.

474 Bacterial community structure and biogeochemical activity in an aquifer contaminated with pesticides

S. Mauffret, BRGM / Environmental geochemistry and water quality; N. BARAN, M. Charron, C. Joulian, BRGM

Our objective was to assess the effect of cocktails of pesticides on groundwater microbial community structure and function. We used two complimentary approaches: a 2- year monitoring at a catchment level (Ariège alluvial plain, France) and microcosm with groundwater collected in 2 parts of the aquifer and then spiked with selected herbicides having a high occurrence in this aquifer, atrazine (ATZ), desethyl atrazine (DEA) and ATZ+DEA. Abundance of the universal marker (16S rRNA) and of nitrate-reducing bacteria (nirG and napA) was assessed by quantitative PCR (qPCR). Diversity was assessed using fingerprinting technique, CE-SSCP (Capillary Electrophoresis–Single Strand Conformational Polymorphism). Pesticides in water were analyzed by LC-MS/MS following an on-line solid phase extraction, and samples for anions and cations were analyzed by ion chromatography. Preliminary results of the microcosm experiment show that biodiversity was higher in water historically contaminated than in pristine-like water and remained higher under laboratory incubations with ATZ, DEA or ATZ+DEA concentrations. Pesticide concentrations in the water historically contaminated often exceeded the legal EU threshold for groundwater and drinking waters (2-year mean: 0.09 ± 0.01 µg ATZ/L, 0.43 ± 0.06 µg DEA/L (n = 23)). On the other side, during microcosm incubation, biodiversity decreased when spiked ATZ, DEA or -ATZ+DEA decreased from 1 to 10 µg/L. The underlying analysis of the two-year monitoring at the catchment level will enable to refine boundaries within this complex relationship between biodiversity and pesticide contamination. ATZ, DEA or ATZ+DEA exhibited similar effect on the microbial community. The nitrate reducing bacterial community strongly decreased during the microcosm experiment. In situ samples were used to clarify the complex interactions between bacteria reducing activity and the possible risk of nitrate accumulation and/or the possible risk of inhibition effect of nitrate on pesticide biodegradation. Biomass was similar in all conditions, suggesting that this is not a sensitive endpoint to assess water quality. This study has the potential to provide sound-based arguments to be considered when improving the current strategy to manage water quality as well as when proposing end point to monitor the microbial community in the biodiversity objective under the European water directive framework.

475 Effects of copper and herbicide mixtures on phytoplankton community from the Charente river estuary

S. Stachowski, IFREMER / BIOECOCHIMIE ECOTOXICOLOGIE; S. Guédon, C. Béchém, L. Haugarreau, D. Ménard, J. Rouxel, IFREMER; N. Coquillé, IRSTEA Bordeaux / Environmet Nantes EPOC (LPTC); G. Vérité, G. Charpentier, J. Seugnet, J. Grizou, J. Chabrand, C. Brach-Papa, J. Chiffoleau, D. Anger, E. Rouzel, IFREMER; C. Lambert, CNRS; P. Soudant, Institut Universitaire Européen de la Mer (IUEM) Université de Bretagne Occidentale (UBO); L. Quiniou, B. Beker, Université de Bretagne Occidentale / Université de Bretagne Occidentale; A. Jadas-Hécat, P. Communal, Université Angers

This field study was conducted in the Charente river estuary (Marennes-Oléron bay, France). The main goal was to assess the impacts of copper and herbicides on phytoplankton communities. Physical, chemical (nutrients, metals and pesticides) and biological (phytoplankton community) parameters were monitored in the field during three years, from 2011 to 2014. In 2012 and 2013, field sampling was coupled with experimental exposure of phytoplankton communities to copper and to an herbicide cocktail, after transportation to the lab: the effects were assessed using photosynthesis endpoint and cellular densities. This study described the framework of Charente estuary contamination with pesticides: herbicides glyphosate and metolachlor were almost permanently detected at the most upstream location. The most elevated concentrations were noticed during the first part of each year. Samples from spring and early summer also exhibited other herbicides: dimethamid, acetochlor, bentazon, mesotrion, metamitron. Phytoplankton dynamics in the natural environment was studied as a whole but also on community subsets: micro-, nano-, pico-phytoplankton and cyanobacteria Synechococcus sp.. This study highlighted the influence of copper and PAHs on the whole phytoplankton community abundance was shown to be temperature, related to seasons; however other variables were also significant drivers for the community subsets. The analysis model used highlighted an abnormal low abundance period for Synechococcus sp. during spring 2012. At the same time, a massive freshwater discharge that brought pesticides was also noticed: although this event was not identified as responsible for this drop, it might have weakened the community. Laboratory experiments showed that a high sensitivity of phytoplankton communities that were exposed to copper in 2012 especially, Synechococcus sp. were shown to be impacted by copper, at environmental concentrations at the μg.L⁻¹ level. The herbicide cocktail used in 2012 (glyphosate, metolachlor and mesotrion) induced no significant effect on phytoplankton communities, whatever the concentration tested and the season. In 2013, the modified cocktail (glyphosate, S-metolachlor, dimethamid and metamitron) led to significant but slight effects on photosynthesis with no variation related to stations or seasons. Tests run from spring to autumn during two years did not demonstrate phytoplankton sensitivity towards the tested herbicides at environmental relevant concentrations.
flamingo parasites *Flamingopiles liguolodes* and that of Gebre parasite *Conflatuaria podicipina* affected the degree of resistance to As. An increase of 4°C temperature as expected by climate change scenarios strongly decreased resistance to Arsenic in both groups, with a stronger effect in uninfected individuals. We explain our results on the basis of the effect of parasites on the canthaxanthins levels of hosts, which are known to have a potent antioxidant and detoxification effect. Our results highlight the importance of considering the infection level of aquatic organisms in ecotoxicological studies to obtain reliable conclusions.

**Innovations in environmental analytical chemistry: the quest for pollutants using target and non-target approaches (II)**

477 Profiling of emerging contaminants in drinking water using passive samplers A.K. Zenker, University of Appl. Sc. Northwestern Switzerland / Institute of Ecopreneurship; T. Hettich, G. Schlotterbeck, University of Applied Sciences Northwestern Switzerland / Institute of Chemistry and Bioanalytics. Application of passive sampling techniques to determine the major fractions of polar and nonpolar contaminants in the quality of the drinking water. Potentially a broad spectrum of chemicals is expected to appear, but it is not feasible to analyse all groups of chemicals in a simple targeted method. Water sampling is a limiting factor as it is performed mostly by grabbing water at defined time points providing residue data only for the moment the sample was taken. Many emerging contaminants are therefore not detected in the water sample. In contrast passive integrative samplers collect a broad range of chemicals over one or two months. The aim of this study was to establish a workflow for a non-targeted profiling of emerging contaminants in drinking water in which polar organic chemical integrative sampler (POCIS) were combined with liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Beside our routinely identified micropolutants in target screening on a HPLC-ESI-QqQ-MS/MS, aliquots of POCIS extracts were also measured in non-target screening on a HPLC-ESI-Q-TOF-MS in positive and negative mode. The separation was done on a Zorbax Eclipse Plus C8 column in gradient. Mass spectra were acquired in scan mode from 100-1000 m/z and with 0 CID voltage. The scan data were matched against in-house compound library to annotate substances in the extract. For further comparison of different sampling sites the data's were processed with statistical analysis (Agilent Mass Profiler Professional, 12.6). Significant separating features identified from principal component analysis were selected to create a targeted hit list. In the second level the extracts were analysed with the hit list in fragmentation mode by 10, 20 and 40 CID V to obtain structural information. These MS/MS spectra's were matched against focused databases to identify compounds. In addition, it was possible to perform de novo identification by try and error. The complexing agent, Ethylene-diaminetetraacetic acid, the halogenated hydrocarbon, Tetrachloroethene, and radiocontrast agents Lopamidol and Ioxitalamic acid, were detected in concentrations higher than 10 ng/L.

478 Profication, characterization and ecotoxicity of disperse azo dyes G.d. Umbuzeiro, FACULTY OF TECHNOLOGY - UNICAMP / LEAL; M. Szynyczek, M. Li, Y. Chen, NCUS / College of Textiles; I.A. Vendematti, A.F. Albuquerque, University of Campinas; A. Santos, FACULTY OF TECHNOLOGY UNICAMP / LEAL Laboratory of Ecotoxicology and Environmental Microbiology; B.S. Maselli, SCHOOL OF TECHNOLOGY UNICAMP / LEAL Laboratory of Ecotoxicology and Environmental Microbiology; F. Kummrow, Universidade Federal de São Paulo; N.R. Vinueza, NCUS / College of Textiles; H. Freeman, North Carolina State University

Although tons of disperse azo dyes have been used worldwide, little information regarding their ecotoxicity is available. Because dyes are subject to regulation under the REACH program there is an urgent need for ecotoxicity data. However regarding their ecotoxicity is available. Because dyes are subject to regulation.

**479 Advances in trace detection, quantification, and partitioning property estimation of biocides with high resolution mass spectrometry (HRMS)** J.S. Ayre, Architecture Civil and Environmental Engineering; S. Samanipour, EPFL, D. Nabi, Masdar Institute / iWater; P. Dimitriou-Christidis, EPFL, Switzerland / LMCE, IEE; J. Gros, LMCE

Evolution imaging of technical products continues to introduce new halogenated chemicals into the environment that remain un-evaluated for their bioaccumulation potential, persistence, and toxicity. These chemicals may be overlooked by existing analytical techniques that must detect and quantify trace analytes despite substantial background signal arising from matrix. Additionally, conventional sample clean-up steps, often employed to decrease signal from matrix, may remove analytes of interest. Comprehensive two-dimensional gas chromatography, or GCxGC, is increasingly recognized as an effective analytical tool for separating complex mixtures of nonpolar contaminants. However, the very rich signal data produced by GCxGC is challenging to analyze and remains largely under-exploited. In particular, limited efforts have been made to detect and quantify trace analytes or conduct suspect screening with GCxGC, in real environmental samples. I will report on several recent advances in our laboratory, including: (i) improved detection, quantification, and tracking of overlooked halogenated pollutants at trace levels using high-resolution mass spectrometry (HRMS); (ii) the use of two complementary tools for screening of organic pollutants in river water and evaluation of treatment efficiency J. BARRITAUD, VEOLIA Recherche et Innovation (VERI); C. Tondelier, V. Ingrand, VEOLIA Recherche et Innovation; S. THIBERT, SEDIF Water sampling is a limiting factor as it is performed mostly by grabbing water at defined time points providing residue data only for the moment the sample was taken. Many emerging contaminants are therefore not detected in the water sample. In contrast passive integrative samplers collect a broad range of chemicals over one or two months. The aim of this study was to establish a workflow for a non-targeted profiling of emerging contaminants in drinking water in which polar organic chemical integrative sampler (POCIS) were combined with liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Beside our routinely identified micropolutants in target screening on a HPLC-ESI-QqQ-MS/MS, aliquots of POCIS extracts were also measured in non-target screening on a HPLC-ESI-Q-TOF-MS in positive and negative mode. The separation was done on a Zorbax Eclipse Plus C8 column in gradient. Mass spectra were acquired in scan mode from 100-1000 m/z and with 0 CID voltage. The scan data were matched against in-house compound library to annotate substances in the extract. For further comparison of different sampling sites the data's were processed with statistical analysis (Agilent Mass Profiler Professional, 12.6). Significant separating features identified from principal component analysis were selected to create a targeted hit list. In the second level the extracts were analysed with the hit list in fragmentation mode by 10, 20 and 40 CID V to obtain structural information. These MS/MS spectra's were matched against focused databases to identify compounds. In addition, it was possible to perform de novo identification by try and error. The complexing agent, Ethylene-diaminetetraacetic acid, the halogenated hydrocarbon, Tetrachloroethene, and radiocontrast agents Lopamidol and Ioxitalamic acid, were detected in concentrations higher than 10 ng/L.

480 LC-HRMS and GCxGC-TOF-MS: two complementary tools for screening of organic pollutants in river water and evaluation of treatment efficiency J. BARRITAUD, VEOLIA Recherche et Innovation (VERI); C. Tondelier, V. Ingrand, VEOLIA Recherche et Innovation; S. THIBERT, SEDIF Water sampling is a limiting factor as it is performed mostly by grabbing water at defined time points providing residue data only for the moment the sample was taken. Many emerging contaminants are therefore not detected in the water sample. In contrast passive integrative samplers collect a broad range of chemicals over one or two months. The aim of this study was to establish a workflow for a non-targeted profiling of emerging contaminants in drinking water in which polar organic chemical integrative sampler (POCIS) were combined with liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Beside our routinely identified micropolutants in target screening on a HPLC-ESI-QqQ-MS/MS, aliquots of POCIS extracts were also measured in non-target screening on a HPLC-ESI-Q-TOF-MS in positive and negative mode. The separation was done on a Zorbax Eclipse Plus C8 column in gradient. Mass spectra were acquired in scan mode from 100-1000 m/z and with 0 CID voltage. The scan data were matched against in-house compound library to annotate substances in the extract. For further comparison of different sampling sites the data's were processed with statistical analysis (Agilent Mass Profiler Professional, 12.6). Significant separating features identified from principal component analysis were selected to create a targeted hit list. In the second level the extracts were analysed with the hit list in fragmentation mode by 10, 20 and 40 CID V to obtain structural information. These MS/MS spectra's were matched against focused databases to identify compounds. In addition, it was possible to perform de novo identification by try and error. The complexing agent, Ethylene-diaminetetraacetic acid, the halogenated hydrocarbon, Tetrachloroethene, and radiocontrast agents Lopamidol and Ioxitalamic acid, were detected in concentrations higher than 10 ng/L.

481 Accurate Mass Spectrometry as a Tool for Characterization of Complex Lysimeter Leachate Samples J. L. Hang, Syngenta Limited / Product Metabolism; S.J. Marshall, Syngenta Ltd / Product Safety; M. Saeed, Smithers Viscient; M. Earll, Syngenta Ltd / Product Safety; L.H. Hand, Veolia Water Solutions / Ingrand, VEOLIA Recherche et Innovation; S. THIBERT, SEDIF Evaluation of water contamination wether it be river water or drinking water is traditionally performed with targeted analyses that measure a limited number of well known molecules usually regulated. Even if these analyses are compulsory they only give a restricted view of pollutants in water samples. New approaches are necessary to identify emerging compounds and evaluate the global quality of water. With that focus, a screening methodology using two complementary analytical techniques was developed by VERI. Apolar to semi-polar compounds are extracted with the sensitive Stir Bar Sorptive Extraction (SBSE) and analysed by in-line thermal desorption and comprehensive two-dimensional gas chromatography coupled with time of flight mass spectrometry (GCxGC-TOF-MS). Semi-polar to polar compounds are extracted with a large scale Solid Phase Extraction (SPE) protocol and analysed on a liquid chromatography coupled with a LQT–Orbitrap high resolution mass spectrometer (LC-HRMS). The enableable rapid and sensitive screening halogenated pollutants by use of GCxGC coupled to high mass accuracy electron capture negative chemical ionization – time–of–flight mass spectrometry (ENCI–TOFMS), a prototype instrument; and (iii) estimation of environmental partitioning properties of nonpolar and polar compounds based on their GCxGC retention times, which then enables rapid and sensitive assessment of nonpolar analyte environmental behavior and bio-partitioning. Application of these techniques successfully elucidates several less-studied halogenated pollutants that occur in the aquatic environment and that warrant prompt investigation.
which has a complex metabolic pathway, with many opportunities for transient intermediates. Quantification of the radioactivity in the leachate suggests that >100 radioactive components are present at concentrations of >0.1 µg/L. According to SANCO guidance, any of these components which are true metabolites of S-metolachlor must be identified in order to assess them for relevance. However, it is not possible to identify all metabolites within the sample conclusively, as there is insufficient metabolite mass in the sample for the NMR analysis that would be required. Recent advances in accurate mass spectrometry allows innovative screening approaches to characterize the content of complex leachate samples to a higher degree than previously possible. Leachate from a lysimeter study was screened for accurate masses (±5 ppm) corresponding to over 400 “theoretical” metabolite structures (permutations and combinations of the known functionality in metabolites previously identified in regulatory studies and published literature). Radio-HPLC analysis was also performed to allow quantification. “Theoretical” metabolites which were not present at >0.1 µg/L were then removed from the metabolite list. Iterative interrogation of the LC-MS data (along with chemical characterization of the leachate) was also conducted to allow further refinement of the hypothetical metabolite list to a shorter “plausible” metabolite list. These refinement steps resulted in a smaller list of 130 “plausible” metabolites, representing a comprehensive description of the most likely metabolites present. Each was then assigned to a chemical cluster based on the functional groups present and a Principal Component Analysis model was developed based on molecular descriptors to visualise the entire “chemical space” and validate the assignment of metabolites to clusters. This characterization and PCA validation can assist with the selection of exemplar metabolites for assessment according to the SANCO relevance guideline, which represent the leachate as a whole.

482 Suspect screening of REACH’S high production volume chemicals in environmental samples


Chemicals’ legislation in the European Union aims to control risks to humans and the environment to ensure safe use of chemicals. Currently, the assessment of chemicals is based on the ratio of predicted environmental concentrations (PEC) and predicted no effect concentrations (PNEC). The objective of this study is to test if and how broad screening analyses of environmental samples can be applied to provide additional information on occurrence of chemicals for further evaluation or risk management. Based on broad screening data of environmental samples, obtained with liquid chromatography coupled to high resolution accurate mass spectrometry were matched with 2402 chemicals with 1846 unique elemental compositions (suspect screening). The selected chemicals had production volumes of 100-1000 tons or were part of priority lists due to their persistence, bioaccumulation or toxicity (PBT) or carcinogenicity, mutagenicity or reproductive toxicity (CMR). The selected chemicals were matched, chemicals could not be identified. Nevertheless, the applied suspect screening can (1) guide further evaluation of chemicals by triggering in depth study of REACH dossiers as delivered by industry as well as (2) providing a selection of chemicals to be identified. Thereby suspect screening can support risk assessment and management of these high production volume chemicals and guide monitoring.

Challenges in Wastewater Treatment and Reuse and the Agricultural Use of Manures and Biosolids (II)

483 Evaluating the potential of bacterial biodegradation of sulfonamides to improve wastewater processing

B.A. Kolvenbach, University of Applied Sciences and Arts Northwestern Switzerland / Institute for Ecoproduction; B. Ricken, University of Applied Sciences Northwestern Switzerland / Institute for Ecoproduction; P. Kei, M. Reis, A.C. Reis, Faculdade de Engenharia da Universidade do Porto / Laboratório de Engenharia de Processos Ambiente Biotecnologia e Energia; C.M. Mauha, CBQF Centre of Biotechnological Research; C.A. Fina, O.C. Noins, Faculdade de Engenharia - Universidade do Porto / LEPABE; Laboratório de Engenharia de Processos Ambiente Biotechnológica e Energia; P.F. Corvini, University of Applied Sciences and Arts Northwestern Switzerland (FHNW)

Antibiotic residues have been detected in wastewater worldwide. Sulfonamides are among the most prescribed antibiotics in human and veterinary medicine. Following intake, they are mainly released without modifications. The presence of antibiotic residues in wastewater has been shown to correlate with the prevalence of antibiotic resistant bacteria. Hence, measures to reduce the load of these chemicals and biological micro-pollutants are needed if the reuse of wastewater is envisaged. Up to now, advanced chemical oxidation methods have been shown to efficiently remove antibiotic residues from wastewater. However, biological degradation systems are described has being cost effective. However, with the exception of beta-lactamases, the information on the enzymatic cleavage of antibiotics is scarce. Up to now, only few sulfonamide degrading bacteria have been isolated, most of which were only found recently. Pseudomonas psychrophila strain HA-4/Jiang et al. 2014; Actinobacteria strain BR1 (Ricken et al. 2013) and Achromobacter sp. strain PR1 (Reis et al. 2014) are among them. The present study is focused on these last two phylogenetically distant bacteria (of the phyla of Actinobacteria and Proteobacteria, respectively). Both strains, isolated from wastewater, are resistant to sulfamethoxazole (SMX), harboring genes encoding sulfonamide resistance (sul1 gene). They release 3-amino-5-methylisoxazole as a dead-end-metabolite during SMX degradation. Furthermore, besides SMX, both can degrade several other sulfonamides, with the release of 3-amino-5-methylisoxazole as metabolite resultant of sulfamethoxazole breakdown. A comparison of the sulfonamide degradation pathway in these two phylogenetically distant bacteria can give first insights into the evolution of this rare biodegradation process. These bacteria or their enzymes can be valuable bioremediation tools contributing to improve wastewater treatments. From a metabolic point of view, the study is also important to determine the role of this degradation mechanism in the spread of antibiotic resistance in order to design strategies that minimize the risk of its dissemination. References Jiang B, et al. 2014. Appl Microbiol Biotechnol 98:4671–4681. Ricken B, et al. 2013. Appl Environ Microbiol 79(18):5550-5558. Reis PJM, et al. 2014. J Hazard Mater 260:741–749.

484 Tuning Microbial Function to Transform Micropolutants during Managed Aquifer Recharge and Engineered Biofiltration

J.E. Drewes, Technische Universität München / Chair of Urban Water Systems Engineering

Attenuation of micropolutants during managed aquifer recharge (MAR) is mainly caused by microbial transformation. Though the underlying mechanisms and selective variables that determine biotransformation are not yet well understood, prior work has revealed that oligotrophic conditions resulted in a more diverse microbial community, which can feature the ability to transform less biodegradable micropolutants. The aim of this high-throughput metabolomic approach was to identify how these factors could be implemented in new treatment strategies for managed aquifer recharge with recycled water to fully utilize the metabolic potential of the microbiome to transform micropolutants. To facilitate the development of these new approaches, we paired removal studies of select micropolutants in controlled laboratory-scale systems and full-scale MAR sites, state-of-the-art chemical water treatments (LC-MS/MS), high throughput sequencing, and biological microarray analysis. The results indicate that low BDOC improves removal efficiency of micropolutants. The metabolic capabilities of microbiome involved in biodegradation were significantly promoted under lower BDOC concentration and higher humic acid content. Cytochrome P450 genes were also more abundant under these primary substrate conditions promoting attenuation of most micropolutants. Full-scale validation studies were performed at a MAR site that had established sequential groundwater recharge systems, that exhibited oxic, oligotrophic conditions. Results are illustrated in Figure 1 revealing a significant increase in performance under these conditions. Findings of this study suggest that very promising opportunities exist to modify design and operation of managed aquifer recharge or engineered biofiltration facilities to select for those microorganisms that can transform even moderately degradable micropolutants more efficiently. [1] Li, D., et al. Applied and Environmental Microbiology 2012,78(19), 6819-6828. [2] Li, D., et al. Water Research 2013,47,2421-2430. [3] Li, D., et al. 2014, Appl. Microbiol. Biotechnol. 98, 5747–5756. [4] Alidina, M., et al. (2014). J. Environmental Management 144, 58-66. [5] Alidina, M., et al. (2014). Water Research 56, 172-180.

485 Urban Wastewater Management, Combined Sewers, and Antibiotic Resistance Dissemination

M. Quintela Baluja, Newcastle University / Environmental Engineering; D.W. Graham, Newcastle University / Civil Engineering and Geosciences; J. López, Romalde, University of Santiago de Compostela / Department of Microbiology and Parasitology

Antibiotic resistance (AR) is an evolutionary response in bacteria to antibiotics, which has become a serious worldwide public health concern with antibiotic resistance genes (ARGs) now considered by many as emerging pollutants. Consequently, WHO has appealed for urgent concerted actions by governments, health professionals, industry, and civil society to (i) slow the spread of drug resistance, (ii) mitigate the potential fallout from AR outbreaks, and (iii) preserve...
medical advances for future generations. This project evaluated urban sewage management systems and how they affect the dispersion of ARGs to the environment. We specifically monitored a waste treatment plant (WTP) in Northern Spain, which included primary and secondary treatment (conventional activated sludge), and river water above and below the WTP in September 2014. Influent, effluent and intermediate locations in the WTP and also sediment and water column samples were collected and analysed using different culturing media, including: MacConkey agar and Eosin Methyl Blue (E.M.B) agar, deoxycholate citrate agar (D.C.) agar, and chromogenic agars designed to detect extended-spectrum β-lactamase (chromID ESBL, bioMérieux) and carbapenem (chromID CARBA, bioMérieux) resistant gram-negative bacteria (total and thermotolerant). Results show that unit operations in the WTP efficiently reduces the total number of bacteria as well as resistant bacteria, especially total coliforms, in final effluent, but showed bacterial increases in the waste sludge. However, thermotolerant bacteria displayed a different pattern, decreasing both through the WTP and also sludge samples. Although the WTP seemed to be reducing ARB in the treatment processes, very high levels of ARB were found downstream WTP. Further examination showed that this was likely because the wastewater inputs to the WTP were in combined sewers, which were overloaded during storms and transiently releasing untreated sewage directly to the river. Our results suggest that part of the solution to reducing ARB and ARB from wastes is modernising the urban wastewater system, specifically reducing combined sewers for sewage carriage. This has particular significant to emerging countries as they build and develop new wastewater collection systems. AR is a global problem that affects developing and developed countries, but other factors such as how sewage in transported must also be considered in new management systems.

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Quantitative analysis of 296 antibiotic resistance and related genes in an urban wastewater treatment plant

A. Vinals, Department of Food and Environmental Sciences; T. A. Johnson, Michigan State University / Center for Microbial Ecology Department of Civil and Environmental Engineering; K. Pärnänen, C. Lyra, M. Tamminen, University of Helsinki; R. D. Steffid, J. M. Tiedje, Michigan State University / Center for Microbial Ecology Department of Civil and Environmental Engineering; M. Virta, Wastewater treatment plants (WWTPs) receive sewage from various sources, including hospitals and households which are both important sources of antibiotics and their residues, and antibiotic resistant bacteria. Bacteria from various environments are mixed in WWTPs and therefore these facilities are considered to be important hot-spots of antibiotic resistance and spread of resistance genes. In this study we quantified genes related to antibiotic resistance (AR) and horizontal gene transfer (HGT) in inflow, effluent and activated sludge over four seasons during one year. The purification process in the plant is based on activated sludge followed by biological post-filtration. In addition the effect of the treated waste water discharge was studied by comparing the sediment near the release pipe with control sediments without direct impact of waste water. The genes were quantified using parallel qPCR array targeting 296 genes (286 ARGs, 9 transposase genes and 16S rRNA gene). Water samples were obtained from Viikinmäki WWTP (Helsinki, Finland) as 24 hour composite samples. Sediment samples were taken from surface sediments. DNA was isolated by using MoBio DNA isolation kits (MoBio Laboratories, Inc., CA, USA). The genes were quantified using highly parallel qPCR array on Applied Biosystems OpenArray platform and normalised with 16S rRNA gene numbers. From the studied 295 AR genes and 16S rRNA genes 184 were found from the WWTP. The mean AR gene numbers in the water contained the highest number of ARGs, followed by the final effluent and sludge. In the sediments near the release pipe the ARG richness was even lower than in the control sediments. All the different ARG classes and every HGT related gene targeted by the assay were found from the WWTP. The mean ARG richness in the raw inflow was 156 genes (min: 149, max: 165), in the final effluents 70 genes (min: 43, max: 101), in the sludge 55 genes (min: 43, max: 67), in the release site sediment 34 genes (min: 25, max: 42) and in the control sediment 39 genes (min: 24, max: 49). The raw inflow had higher abundance of ARGs compared to both final effluent and sludge (p<0.001). The ARG abundance in the sediments near the release pipe was not significantly different from the control sediments. Our results demonstrate that urban waste water plant is not necessarily a hot spot of the dissemination of antibiotic resistance, even when based solely on biological treatment.

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Antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the aquatic environment

S. Rodríguez-Mozaga, Institute for Water Research (ICRA) / Water Quality; S. Chamorro, Catalan Institute for Water Research ICRA; E. Martí, Catalan Institute for Water Research (ICRA); B. Huerta Buitrago, Catalan Institute for Water Research (ICRA) / Department of Water Quality; M. Gros; A. Sanchez-Melsio, Catalan Institute for Water Research ICRA; D. Barceló, IQAB-CSIC / Institute for Environmental Assessment and Water Research; C. Borrego, Catalan Institute for Water Research ICRA; J. Balcazar, Catalan Institute for Water Research (ICRA) Antibiotics are widely consumed drugs administered to treat infectious diseases, and large amounts are released into municipal wastewater due to excessive consumption and disposal of unused antibiotics. Overuse and misuse of antibiotics has led to the emergence of antibiotic-resistant bacteria, compromising the effectiveness of antimicrobial therapy. Aquatic ecosystems are an ideal setting for the acquisition and spread of antibiotic resistance genes (ARGs) due to the continuous release of both antibiotics and antibiotic resistance bacteria into the environment through different routes, such as discharge of wastewater treatment plant (WWTP) effluents. In this work we investigate the pollution level of a broad range of antibiotics (β-lactams, lincosamides, macrolides, quinolones/fluoroquinolones, sulfonamides, tetracyclines, dihydrofolate reductase inhibitors and nitroimidazoles) and ARGs (blaTEM, ermB, qnrS, sulf and tetW), released from hospital and urban wastewater in Girona (NE Spain), their removal through a wastewater treatment plant (WWTP) and their presence in the receiving river water. Several antibiotics were detected in all water samples collected. However, good removal efficiency during WWTP was observed for some antibiotics, most of them were still present in WWTP effluents. Copy numbers of ARGs, such as blaTEM (resistance to β-lactams), qnrS (reduced susceptibility to fluoroquinolones), ermB (resistance to macrolides), sulf (resistance to sulfonamides) and tetW (resistance to tetracyclines), were detected at the highest concentrations in hospital effluent and WWTP influent samples. Although there was a significant reduction in copy numbers of these ARBs in WWTP effluent samples, this reduction was not uniform: Concentrations of ermB and tetW genes decreased during treatment process whereas blaTEM, sulf and qnrS gene copy numbers increased. The incomplete removal of antibiotics and ARGs in WWTP severely affected the receiving river, where both types of emerging contaminants were found. Although hospital effluent had higher concentration, especially in hospital effluent samples. Although good removal efficiency during WWTP was observed for some antibiotics, most of them were still present in WWTP effluents. Copy numbers of ARGs, such as blaTEM (resistance to β-lactams), qnrS (reduced susceptibility to fluoroquinolones), ermB (resistance to macrolides), sulf (resistance to sulfonamides) and tetW (resistance to tetracyclines), were detected at the highest concentrations in hospital effluent and WWTP influent samples. Although there was a significant reduction in copy numbers of these ARBs in WWTP effluent samples, this reduction was not uniform: Concentrations of ermB and tetW genes decreased during treatment process whereas blaTEM, sulf and qnrS gene copy numbers increased. The incomplete removal of antibiotics and ARGs in WWTP severely affected the receiving river, where both types of emerging contaminants were found. Although hospital effluent had higher concentration, especially in hospital effluent samples.
University: T.F. Fernandes, Heriot-Watt University / School of Life Sciences Frameworks commonly used in trace metal ecotoxicology (e.g. biotic ligand model (BLM) and tissue residue approach (TRA)) are based on the established link between uptake, accumulation and toxicity, but similar relationships remain unverified for metal-containing NPs. The present study aimed to (i) characterise the bioaccumulation dynamics of PVP-PEG- and citrate-Ag NPs, in comparison to dissolved Ag, in two moss piglets D. magna and Daphnia magna and (ii) investigate whether parameters of bioavailability and accumulation predict acute toxicity. In both species, uptake rate constants for Ag NPs were generally 2-10 times less than for dissolved Ag and showed significant rank order concordance with acute toxicity across both species (r=0.857, p<0.001, Kendall’s tau coefficient). Ag elimination by L. variegatus fitted a 1-compartment loss model, whereas elimination in D. magna was bi-phasic, comprising fast and slow loss phases. The latter showed consistency with studies that reported dephaps ingestings of NPs, whereas L. variegatus biodegradation parameters indicated that uptake and efflux were primarily determined by the bioavailability of dissolved Ag released by the Ag NPs. Our study shows that principles of BLM and TRA frameworks are confounded by the feeding behaviour of D. magna where the ingestion of Ag nanomaterial ions disrupts the balance between tissue burden and acute toxicity, but such approaches are applicable when accumulation and acute toxicity are linked to ionic concentrations. However, the uptake rate constant, as a parameter of bioavailability that includes all available pathways, could be successful predictor of acute toxicity. KEYWORDS: Ag NPs, Biodegradable nanomaterials, nano-BLM, Tissue Residue Approach. Presentation Preference: Platform Acknowledgement - The authors thank the Heriot-Watt University NanoSafety project. Funding was provided by the US EPA – UK NERC Nanobee project.

490 Concentration and form of copper released into aquatic systems from commercial liquid and micronized pressure treated lumber A.N. Parks, U.S. EPA, Atlantic Ecology Division / Atlantic Ecology Division; M.A. Cashman, US EPA / Atlantic Ecology Division; M.G. Cantwell, Atlantic Ecology Division; D.R. Katz, K.T. Ho, R.M. Burgess, U.S. EPA / Atlantic Ecology Division The fate and effects of pristine engineered metal nanomaterials (ENMs) in simplified systems have been widely studied; however, little is known about the potential release and impact of metal ENMs from consumer goods, especially lumber which has been treated with micronized copper. Micronized copper treatment solutions contain copper complexes predominately in the 10-700 nm size range, and are used in lumber to prevent microbial degradation and fouling. In this work, the general goal was to examine the rate, quantity, and form of copper released from several commercially available pressure-treated lumber samples exposed to an aqueous system at 0, 1, 10, and 30 ppt seawater. Lumber tested included untreated Southern Yellow Pine (SYP) as the control, SYP treated with micronized copper azole (MCA) at 0.96 and 2.4 Kg/m², alkaline copper quaternary (ACQ) at 0.30 and 9.6 Kg/m², and chromated copper arsenate (CCA) at 40 Kg/m². Of the different commercial treatments, only MCA included copper and micronized copper complexes. The experimental system included 2 c m wood cubes cut from the outer surface of the lumber submerged in 250 mL of media (0, 1, 10, and 30 ppt reconstituted seawater) in high density polyethylene bottles, and mixed on a shaker table at 120 rpm for 6 months. Water samples were taken at 8 hours, and on days 1, 2, 7, 14, 28, 133, and 210. Subsamples included unfiltered water, and water filtered through a 0.1 µm polyethersulfone (PES) syringe filter and a 3000 Dalton centrifugal filter, which were analyzed using ICP-AES to determine the total, nanovionic copper (<0.1 µm subsample), and presumably ionic copper (Americamysis bahia) were used to confirm the results of the size-based analyses. Overall these results suggest that the form of copper released from treated lumber is ionic and that little free nanocopper is present. This finding suggests the risk associated with nanoparticle treated lumber can be addressed with conventional existing copper ion modelling approaches.

491 Nanomized titanium dioxide mitigates copper toxicity R. Rosenfeldt, National Institute of Water and Environmental Sciences / Institute for Environmental Sciences; F. Seitz, Inst. for Environmental Sciences / Institute for Environmental Sciences; J.P. Zubrod, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; A. Beckler, Swedish University of Agricultural Sciences (SLU) / Department of Aquatic Sciences and Assessment; S.R. Luerdewald, Institute for Environmental Sciences / Institute for Environmental Sciences; T. Schütz, University of Koblenz-Landau; R. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment. The increasing use of titanium dioxide nanoparticles (nTiO₂) as an ingredient in many consumer products (e.g., paints, textiles and sunscreens) inevitably increases the risk of their unintentional release into aquatic environments. Thus, their interaction with other co-occurring stressors (e.g. heavy metals) seems unavoidable. The nanoparticles could adsorb large amounts of metal ions and hence potentially reduce the concentration of free ions in the water phase, while carrying high loads of them at their surface. The settling nTiO₂ agglomerates – together with the absorbed metal ions – might pose a risk to benthic organisms (e.g. gammarids) in particular. This scenario was assessed by taking advantage of a 2×2 factorial test design, exposing Gammarsus fossarum to nTiO₂ (0 or 2 mg/L) and copper (Cu; 0 or 40 µg/L) in a 24-d lasting bioassay. The endpoints were mortality, leaf consumption, feces/food production, growth rates, and consumption related to physiological fitness. The results showed that nTiO₂ caused no mortality and no reduced leaf consumption, while Cu led to a high mortality of G. fossarum (71%). In addition, Cu caused a 25% reduction in leaf consumption as well as a 30% reduction in feces production compared to the control, respectively. The presence of nTiO₂ mitigated the toxic effects of Cu observed in the absence of nTiO₂. Furthermore, nTiO₂ alone was more than 4-fold reduced and the leaf consumption reached levels comparable to the control. Therefore, potential long term effects of nTiO₂ should be thoroughly investigated, before considering the application of nTiO₂ as a remediation agent for e.g. waste water or highly contaminated surface waters.

492 Designing safer nanomaterials towards a sustainable nanotechnology: a study with hydrophobically modified polyacrylic acid nanomaterials J.A. Tavares, University of Aveiro, Department of Biology & CESAM / Biology; C. Venâncio, Biology; C. Duarte, F. Antunes, University of Coimbra; A.M. Soares, Pelophylax perezi, Aveiro / Department of Biology and CESAM; I. Lopes, University of Aveiro / CESAM Biology Department The growing demand for new and better products led to an exponential development of manufactured nanomaterials (MMN) since the 90’s decade. The current widespread use of MMN in consumer products is promoting the contact between nanomaterials and the environment, which may lead to adverse effects in the latter. The present work aimed at suggesting the most environmental-friendly hydrophobically modified polyacrylic acid (HPMMA) MMN in order to contribute for the European Union agenda on Nanosafety: safe and sustainable nanomaterials and nanotechnology innovations. Six variations of HPMMA MNMs were obtained by modifying the crosslinked conformation, the insertion position and the length of hydrophobic groups. Each variation of HMPMA suspensions were characterised by using light scattering techniques. Conductivity and pH were also measured. To characterise the ecotoxicity of the six MNMs a battery of lethal and sublethal standardised assays were conducted with species from different taxonomic and functional levels to assess different ranges of sensitivity: (i) Vibrio fischeri (Azur, 1998), (ii) Raphidocelis subcapitata and Chlorella vulgaris (OECD, 2006), (iii) Brachyosynus calyciflorus (Microbiotest, 1998), (iv) Daphnia magna (OECD, 2006), and (iv) Pseudoplyxus perezi. EC50 values were calculated for each monitored endpoint. Computed LC and EC values ranged from 9.64 mL/L up to 2000 mL/L for all tested MNMs and species. In general, the most sensitive specie to the tested MNMs was the bacteria Vibrio fischleri, while Brachyosynus calyciflorus and Raphidocelis subcapitata – It is suggested that industries should develop HPMMA with higher hydrophobic groups (shorter R groups) to mitigate the risks of these suspensions and reduce their adverse effects to biota while maintaining its functionalization, thus contributing to a more sustainable development and greener nanotechnology.

493 Demonstrating approaches to chemically modify the surface of Ag nanoparticles in order to influence their toxicity and biodistribution C. Pang, University Ca'Foscari Venice / Dept of Environmental Sciences Informatics and Statistics; A. Brunelli, Environmental Sciences Informatics and Statistics; W. Tao, Research Center for EcoEnvironmental Sciences Chinese Academy of Sciences / State Key Laboratory of Environmental Chemistry and Ecotoxicology; D. Heistermann, Università degli Studi di Firenze; E. Semenzin, Ca'Foscari University of Venice / Department of Environmental Sciences Informatics and Statistics; A. Marcomini, University of Venice / Department of Environmental Sciences Informatics and Statistics; C. Chen, National Center for Nanoscience and Technology of China / CAS Key Laboratory for Biomedical Effects of Nanomaterials Nanosafety; B. Zhao, RCIES, CAS AgNPs, PVP AgNPs and BPEI AgNPs, respectively. Our results demonstrated that the toxicity of AgNPs depends on the intracelalaluric localization that was highly dependent on the surface charge. BPEI AgNPs (ζ potential = +46.5 mV) induced the highest cytotoxicity (EC50=10.38 µg/ml) and DNA fragmentation in Hepa1c1c7. In addition, it showed the highest damage to the nucleus of liver cells in
the exposed mice, which is associated with the highest cellular uptake and high accumulation in liver tissues. The near neutral PEG AgNPs (t1/2 = 1.62 h) showed the lowest toxicity (EC50 = 63.14 µg/ml), a long blood circulation (t1/2 = 1.04 h, t1/2d = 436.67 h), as well as a high bioaccumulation in spleen (34.33 µg/g), which suggest better biocompatibility compared to the other chemically modified AgNP. Moreover, the adsorption ability with bovine serum albumin revealed that the AgNP t1/2 is only when PAgNPs consumed in household for efficiently resist opsonization or non-specific binding to prolonged mice. The overall results indicated that the toxicity of AgNPs was significantly dependent on surface chemistry. BPEI AgNPs > Citrate AgNPs = PVP AgNPs > PEG AgNPs. This kind of toxicological data could be useful in supporting the development of safe by design AgNPs for consumer products and drug delivery applications.

Challenges of regional and global modelling nitrogen and phosphorus in agriculture supply chains

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The importance and challenge for accounting of soil stock changes in N footprint and N budget studies
A. Leip, Institute for Environment and Sustainability, F. Ozbek, European Commission Joint Research Centre / Institute for Environment and Sustainability; O. Hutton, University of Virginia / Department of Environmental Sciences Engineering; A. Leach, J. Galloway, University of Virginia / Department of Environmental Sciences

Nitrogen Use Efficiency (NUE) and the Nitrogen Balance (NB) are key indicators assessing the sustainability of agricultural production. When determining NUE or NB for regions or countries on the basis of standard methodologies, accumulation of nitrogen in soils or depletion of nitrogen from soil resources is usually left out of the equation. Yet, soil stock changes might be important elements for the NUE or NB of a crop, with accumulations of N in the soil increasing the NUE and reducing the NB and the inverse effect when soil is losing N from its organic matter (1, 2). For Tanzania, Hutton et al. (3) propose the estimation of the “Soil Mining Factor” (SMF), based on the observation that the majority of fields (91%) in Tanzania were unfertilized and produced only half as much grain than on fertilized fields. This was used to calculate that 83% of N in harvested grains in Tanzania originates from soil resources. SMF as kg N per kg of household sewage (or vegetable) products in Tanzania range between 1.1 and for wheat and 1.9 for vegetables. For Turkey, Ozbek and Leip (4) propose a methodology based on the observation that for many countries the NB method yields very low (or even negative) N surplus, with unrealistically high NUE values. Using detailed NB data for 26 administrative regions in Turkey they developed a hyperbolic regression model estimating a relationship between NB and the NB thresholds. Efforts are needed to provide estimates for nitrogen soil balances for many countries the NB method yields very low (or even negative) N surplus, which is associated with the highest cellular uptake and high accumulation in liver tissues. The near neutral PEG AgNPs (t1/2 = 1.62 h) showed the lowest toxicity (EC50 = 63.14 µg/ml), a long blood circulation (t1/2 = 1.04 h, t1/2d = 436.67 h), as well as a high bioaccumulation in spleen (34.33 µg/g), which suggest better biocompatibility compared to the other chemically modified AgNP. Moreover, the adsorption ability with bovine serum albumin revealed that the AgNP t1/2 is only when PAgNPs consumed in household for efficiently resist opsonization or non-specific binding to prolonged mice. The overall results indicated that the toxicity of AgNPs was significantly dependent on surface chemistry. BPEI AgNPs > Citrate AgNPs = PVP AgNPs > PEG AgNPs. This kind of toxicological data could be useful in supporting the development of safe by design AgNPs for consumer products and drug delivery applications.

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analysis highlighted the importance of regionalising both emissions and characterisation factors. Therefore, we strongly recommend using the crop-specific, regionalised results of this study.

498 Substance Flow Analysis on Agricultural Nutrients focusing on Nitrogen and Phosphorus 
T. Yamamoto, K. Matsubae, Tohoku University; K. Nakajima, National Institute of Environment Studies; K. Nansai, Nat. Inst. of Env. Studies (NIES); T. Nagasaka, Tohoku University

Agricultural nutrient such as phosphorus, potassium and nitrogen are essential elements for food production. These elements are called three major elements of fertilizer, and the demand of them has been increasing not only for agricultural industry but also for other industries. In 2050, the world population will reach more than 9 billion and stable food supply is one of the most important issues. Furthermore, amount of bioethanol production has increased about three times in the past ten years. From these reasons, the demand of the three elements will increase as well as that of fertilizer. The aims of this study were the following three, (1) to grasp supply and demand of nitrogen and phosphorus using material flow analysis; (2) to estimate global phosphorus and nitrogen flows associated with export and import of various commodities; and (3) to analyze phosphorus and nitrogen consumption between the Japanese domestic economy and the global supply chain using the GLIO model. Furthermore, we have quantitatively clarified agricultural nutrients utilisation for various countries and discussed the possibilities and bottlenecks of recovery and recycling of N and P from the waste streams. The method of this study was threefold, (1) Material flow analysis of nitrogen and phosphorus subject to 2005 in Japan. (2) The estimation of nitrogen and phosphorus accompanied with each item traded among 231 countries and regions basis of BACI. 423 types of N and 281 types for P contained products were chosen from HS code. (3) The agricultural nutrient flows into the 2005 input-output model of the Japanese economy, and integrates international trade statistics on the basis of Global Linkage Input Output (GLIO) model. The result of this study about nitrogen, North America and China are the key stakeholders, which have large amount of excess balance of nitrogen. About phosphorus, North and South America and China have large amount of excess balance of phosphorus, while most African countries, even P ore mining countries, face on the low balance of P. From the estimated results, nitrogen and phosphorus are flow in as fertilizer and feed, and mostly ended up to flow out the environment in the developed countries. It is expected that the consumption of nitrogen and phosphorus will increase as the economic development in BRICS and VISTA. The more effective utilisation of these nutrients and the geopolitical and environmental governances are strongly required in coming decades.

Prospective Life Cycle Thinking approaches for the definition and implementation of sustainability strategies in industry and policy making (II)

499 How much biochar does gasification bioenergy need to be carbon neutral?
K. Saez de Biküha, Chemical Biochemical Engineering; A. Broom, DTU Technical University of Denmark / Chemical and Biochemical Engineering; M. Hauchild, DTU Management Engineering

Indirect land use changes (iLUC) from bioenergy emerge whenever an energy crop is planted on arable land [1]. Due to their overarching magnitude from a life-cycle perspective, they have repeatedly been recommended to be included in bioenergy’s greenhouse gas (GHG) accounting, despite their challenging quantification and inherent uncertainties [2], [3]. Marginal or abandoned lands have been often quoted as the solution to avoid these undesired effects from bioenergy [4], [5]. However, land abandonment and marginalization is to a large extent a socio-economic process, and thus heavily depends on specific, constantly changing socio-political conditions. In other words, it is in place [6]. We suggest a carbon negative bioenergy system that compensates for potential iLUC emissions and losses in soil organic carbon (SOC). A consequential life cycle assessment on willow bioenergy has been performed, distinguishing marginal and arable land scenarios. Specific soil types and their estimated SOC changes have been considered [9], as well as iLUC emissions for the arable case [10]. Taking the study case of a willow plantation combined with a medium-scale gasification plant in Denmark, we illustrate the biochar needed from the process in order to remain carbon neutral. The time scopes assessed are 20 and 100 years and it is assumed a fossil fuel (FF) free Denmark beyond 2050 as targeted by government (no FF displacement occurs after 2050). Results show that willow on marginal land remains carbon negative (4% higher CO2 equivalents than in the short run), while a willow biochar fraction of 31% of biomass (i.e., 6.4 g Mg C ha\(^{-1}\) yr\(^{-1}\) would be necessary in 100 years to be carbon neutral (taking natural vegetation as reference baseline). As for arable land willow, a biochar fraction of 34.1% (or 2.32 Mg C ha\(^{-1}\) yr\(^{-1}\)) would be necessary in the short term to compensate for iLUC emissions, while a 4% would suffice to make it carbon negative in the long term, as iLUC “dilutes” over 100 years. To achieve such high biochar fractions (>10%), lower process temperatures are needed, which affect negatively the long-term stability of biochar [11], [12]. This can put at risk the claimed GWP reduction benefits. This study did not consider impacts on other environmental aspects as ecosystem services and biodiversity, which are deemed to be rather important and significant for iLUC.

500 LCA of alumina nanofluid used as coolant in Power Electronic Traction system
S. Scalbi, P. Masoni, ENEA / LCA and Ecodesign Laboratory

Nowadays the emerging nanomaterials are spreading in several markets, so the potential environmental impacts evaluation is essential to avoid undesirable effects on environment. The study aim is to analyse from cradle to grave the environmental impact of the alumina nanofluid (NF) used as coolant in the cold plate of the Inertial Gate Bipolar Transistor (IGBT). This is a modern device used in Power Electronic Traction system (PET). The main problem in PET is the effective temperature management because the efficiency depends on the cooling system. A comparative LCA was developed to study the environmental impacts of three systems: 1. Baseline Cooling System (BCS) using water and glycol as refrigerant; 2. Nanofluid Cooling System single stage (NCS single) using alumina NF refrigerant produced with the single-stage process (wet chemical synthesis to form and disperse tailored nanoparticles within a fluid); 3. Nanofluid Cooling System two stage (NCS two) using alumina NF produced with the two-stage process (batch process added pre-produced nanoparticles to a fluid). The system function is removing heat from PET in order to improve its performances and lifetime in trains. The functional unit is a liquid cooling system for PET, capable to keep specified performance and with in one of the key technological transitions of our time and this study analyzes implications, from a metallic resources availability perspective, of international policymaking goals for worldwide introduction of electric vehicles (EVs) by 2020 and 2030. To meet 2020 and 2030 policies, an introduction and maintain of an EV fleet of 3 800 000 or 15 million vehicles is required. On one hand, the amounts of resources needed were assessed and compared to the overall global availability and primary production as on one the other hand, the sensitivity of the MACSI, a competition factor used as LCIA midpoint indicator for the evaluation of resource depletion, to a major technological shift was tested. A general explanation on how MACSI is calculated to assess resource depletion is also shown. Resources for which average annual production needs to be increased significantly (>1%) are lithium and cobalt when EVs are Li-ion battery powered and nickel, rare earths and lithium when EVs are NiMH battery powered. Although production needs to be increased for these resources, the competition factor is quite stable in terms of resource ranking when introducing such a key technological shift. Along the electrification scenario of meeting and maintaining the 2020 and 2030 goals, a total worldwide replacement of the light-duty fleet (i.e. 750 million vehicles) scenario was imagined. When considering this global transition to EVs, cobalt, copper and lithium show a very significant increase in competition factors (variations of 87%, 58% and 71%, respectively) and an important reduction in the number of years before base depletion (variations of 81%, 13% and 94%, respectively). Therefore, although competition modeling is a robust indicator when looking at a short term transition, there is a need to update its values and its ranking when a long-term perspective is considered.

502 Using the reliability theory for interpreting comparative LCA
w. wei, Istea Clermont Ferrand; P. Larrey-Lassalle, IRSTEIA Montpellier; T. Faure, LISC IRSTEIA; N. Dumoulin, Istea - Lisc; P.C. Roux, Istea / UMR ITAP ELSA; L. Mathias, Istea Lisc

The interpretation of results is one of the four main phases of LCA according to the ISO standards. It identifies, quantifies, and evaluates information from the results of the Life Cycle Inventory (LCI) and the Life Cycle Impact Assessment (LCIA) in order to provide recommendations that allow decision makers to take future
decisions. However, today LCA still needs robust interpretation methods for addressing results uncertainty. Here we apply an approach called reliability analysis for comparative LCA interpretation. Reliability analysis aims at comparing two products and calculates the failure probability which expresses the probability of a product to have a smaller environmental footprint than that of another product. The failure probability also represents the probability that the decision maker takes a wrong decision. We apply reliability methods to two methods: Monte Carlo simulations and the FORM method. Monte Carlo simulations give us the failure probability (in terms of decision). The FORM method enable us to approximate the failure probability with fewer simulations than Monte Carlo simulations. Moreover, the FORM method calculates the associated important factors (according to the failure probability) which identify a sensitivity analysis in relation to the decision. The important factors provide the sensitivity coefficient to decision makers in order to determine which factors influence their decision. Our results clearly show that the reliability method provides a further useful means of information for the LCA practitioners and decision makers.

503 Assessing and communicating uncertainties in life-cycle based assessments: lessons learned and ways forward
J. Clavreul, Unilever R&D Colworth / SEAC
The assessment of uncertainties and variability in life cycle assessment (LCA) is recognised as a need for making results more robust and strengthening LCA as a key decision making tool. Historically, there are few examples of employing techniques for assessing uncertainty and variability in LCA; however, some practices have become much more common in recent years, thanks mainly to the introduction of the ISO 14044 standard. The techniques used are usually limited to sensitivity analyses which consider one factor at a time but there are high quality examples of the application of more advanced methods in the literature. These techniques include for example the separate reporting of uncertainty and variability, the use of input-output techniques or the use of variance-based sensitivity analysis techniques. They often provide new and valuable insights but their applicability to day-to-day assessments often appears challenging. The author discusses some of the challenges and opportunities involved in assessing and communicating uncertainties for decision support in an industrial context. A review is presented that explores how this can be done in practice for the different types of business decisions encountered in a fast moving consumer goods industry. Using past experience of uncertainty assessment and communication, general guidance is derived on how to represent data quality at various stages of the value chain, aid the interpretation of LCA results and thus enhance evidence based decision making. Based on this experience, a discussion is presented about the major challenge that lies ahead: the communication of uncertainty analysis results in an appropriate form to often non-expert decision makers and stakeholders. Depending on the level of details needed, different approaches can be adopted ranging from incorporating the underlying uncertainty within the assessment result to the communication of complex results via linguistic or graphical mechanisms. Finally ways forward to help strengthen the field of uncertainty and its communication in industry applications are suggested.

Risk assessment, risk management and mitigation for pesticides: from regulation to public perception (II)

504 Effects characterization for aquatic invertebrates exposed to imidacloprid-Part A: A novel evaluation rubric to assess aquatic toxicity studies
L. Knopper; R.L. Breton, Instrinsik Environmental Sciences, Inc.; D. Moore, Instrinsik Environmental, Inc.; T. Hall, Bayer CropScience / Environmental Toxicology and Risk Assessment; L.M. Bowers, Bayer CropScience / Environmental Safety
A review of the scientific literature, registrant-sponsored studies, government agency databases, existing water quality guideline documents and the grey literature revealed 140 studies of the toxicity of imidacloprid to aquatic invertebrates. Tier 1 acute and chronic laboratory studies were in the majority (n=110); the remainder were higher studies (microcosm, mesocosm, rice paddy). Before being used in ecological risk assessment, studies need to be critically evaluated for relevance and quality. We developed an evaluation strategy based on a number of questions (specific to study type and chemical), scored from 0 to 3, to address four assessment factors: utility, objectivity, clarity and transparency, and integrity. A detailed evaluation rubric accompanied the evaluation strategy and provided clear expectations for each score. With this strategy, a study could score between 0 and 26 (Tier I studies based on 15 questions) or 0 and 29 (Tier II studies based on 16 questions). Studies were rated acceptable, supplemental or unacceptable. Studies were split into two groups and reviewed independently by two reviewers. A subset (~30%) of each group was re-evaluated by the opposite reviewer as part of a Quality Assurance/Quality Control process. The first step in the evaluation process was to evaluate data against utility (i.e., relevance) questions. Only studies deemed relevant for risk assessment were further evaluated against the other assessment factors. Of the 110 laboratory studies, 84 were rated as relevant and evaluated for data quality: 25 were rated as acceptable, 27 were rated as supplemental and 22 were rated as unacceptable. Of the 30 higher tier studies, 19 were rated as relevant and evaluated for data quality: 10 were rated as acceptable, eight were rated as supplemental and one study had both acceptable and supplemental data. No higher tier studies were unacceptable. Typically evaluators came to the same numeric score or differed by one or two points, highlighting the transparent, consistent and repeatable nature of this novel evaluation scheme. Details of the evaluation process, specific questions and the scoring rubric will be presented. The strength of the acceptable studies and key data missing for studies that were supplemental and unacceptable will be discussed. The quantitative and qualitative use of supplemental studies as part of the evidence-based risk assessment process will be presented.

505 Aquatic Risk Assessment for Imidacloprid Using All Available Evidence
A. Weyers, Bayer CropScience / Environmental Safety Ecotoxicology; E. Klamroth, Bayer CropScience / Global Regulatory Management; E. Bruns, Bayer CropScience AG / BCS DT EXC Ecotoxicology; C. Garside, Bayer CropScience / Environmental Safety Assessment
For a comprehensive risk assessment for Imidacloprid and aquatic organisms, Bayer Crop Science has generated studies under both laboratory and under more realistic outdoor conditions resulting in a large data base of information. In a recent publication further data were generated under laboratory conditions showing chronic effects for certain species that are lower than were reported before. However, for regulatory purposes these have shortcomings, reported only very briefly, and are partly inconsistent. To address this a new data set was generated. Based on the ISO 14044 standard, the techniques used are usually limited to sensitivity analyses which consider one factor at a time but there are high quality examples of the application of more advanced methods in the literature. These techniques include for example the separate reporting of uncertainty and variability, the use of input-output techniques or the use of variance-based sensitivity analysis techniques. They often provide new and valuable insights but their applicability to day-to-day assessments often appears challenging. The author discusses some of the challenges and opportunities involved in assessing and communicating uncertainties for decision support in an industrial context. A review is presented that explores how this can be done in practice for the different types of business decisions encountered in a fast moving consumer goods industry. Using past experience of uncertainty assessment and communication, general guidance is derived on how to represent data quality at various stages of the value chain, aid the interpretation of LCA results and thus enhance evidence based decision making. Based on this experience, a discussion is presented about the major challenge that lies ahead: the communication of uncertainty analysis results in an appropriate form to often non-expert decision makers and stakeholders. Depending on the level of details needed, different approaches can be adopted ranging from incorporating the underlying uncertainty within the assessment result to the communication of complex results via linguistic or graphical mechanisms. Finally ways forward to help strengthen the field of uncertainty and its communication in industry applications are suggested.
from other insect growth regulators such as chitin synthesis inhibitors and juvenile hormone analogues. Several lab and field studies, including hive feeding studies, have shown low to no effects of methoxyfenozide on honey bee adults and brood. Field exposure studies were conducted to determine potential residues found in hives following applications in blooming orchards. Residues in pollen, nectar and honey bee larvae were quantified. An exposure-based risk assessment comparing exposure to toxicity showed low risk to honey bee colonies following field applications of methoxyfenozide.

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A comparative distribution-based analysis of HC5 values and data from ecological communities results in similar ecotoxological thresholds to concern for PSI1 inhibiting herbicides M. Gustavsson, Department of Biological and Environmental Sciences; L. Posthuma, De Zwart, RIVM / Centre for Sustainability Environment and Health; T. Backhaus, University of Gothenburg / Department of Biological and Environmental Sciences

Species sensitivity distributions (SSDs) and distributions of toxicity values are used for determining ecotoxological and ecotoxological thresholds, the HC5 (hazardous concentration for 5% of the species) and the TTC (threshold of toxicological concern), respectively. An SSD describes the sensitivity distribution of a range of species towards a single toxicant, while the TTC is based on the toxicity distribution of a range of different compounds towards the same species or group of species. Both concepts can be combined by using the distribution of HC5 values for a group of practical implications in order to map the species’ sensitivity as well as TTC values are based on toxicity data from standard single species assays. However, the ecological impact of a compound is the result of the reaction of a range of interacting species. In order to explore the impact of using data from ecological communities instead of single species, we performed a comparison of the TTC based on HC5-values with the TTC based on community ecotoxological data (threshold of concern for community toxicity, TCCT). This study was performed by using toxicity data for Photosystem II inhibiting herbicides to single algal species and algal communities. Single-species based SSDs and the corresponding HC5 values were established for seven different herbicides. Community ecotoxicity data were collected for 17 herbicides. The resulting thresholds were 6.8 mmol/L for the TTC based on HC5 values and 3.5 nmol/L for the TTC based on community data. This indicates that indirect ecological effects and species interactions do not seem to play a major role, as long as photosynthesis is in focus.

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An analysis of the validity of the current risk assessment methodology from regulatory point of view, indicates that it is not sufficiently valid nor reliable, which are fundamental criteria of a scientific method. It is concluded that current methodology is highly complex, flexible, non-standardised and relies to a great extent on expert judgement, which makes conclusions unpredictable, and has practical implications in order to map the species’ sensitivity as well as TTC values are based on toxicity data from standard single species assays. However, the ecological impact of a compound is the result of the reaction of a range of interacting species. In order to explore the impact of using data from ecological communities instead of single species, we performed a comparison of the TTC based on HC5-values with the TTC based on community ecotoxological data (threshold of concern for community toxicity, TCCT). This study was performed by using toxicity data for Photosystem II inhibiting herbicides to single algal species and algal communities. Single-species based SSDs and the corresponding HC5 values were established for seven different herbicides. Community ecotoxicity data were collected for 17 herbicides. The resulting thresholds were 6.8 mmol/L for the TTC based on HC5 values and 3.5 nmol/L for the TTC based on community data. This indicates that indirect ecological effects and species interactions do not seem to play a major role, as long as photosynthesis is in focus.

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Enzymatic degradation of hormones and EDCs in wastewater - a novel biotechnological approach D. Becker, Goethe University Frankfurt / Aquatic Ecotoxicology; R. Schoevarth, Chiralvision; O. Couillerot, J. De Gunzburg, DaVoUterra; M. de Cazes, J. Sanchez-Marcano, M. Belleluie, Institute Européen des Membranes; S. Insa, Institute for Water Research ICRA; M. Llorca, IDAEA, CSIC; S. Rodriguez-Mozaz, Institute for Water Research (ICRA) / Water Quality; D. Ballester, CEN-UB-CSI; INIA and the LO1205 project with financial support

Micropollutants (e.g., pharmaceuticals, hormones) are continuously released into the environment in low concentrations. Concerns have been raised regarding their toxicity and their limited degradation during conventional wastewater treatment. This study presents a novel biotechnological approach to degrade micropollutants. Specific enzyme libraries were screened to identify promising candidates that remove these pollutants effectively. The enzymes are optimized and immobilized for an application in bioreactors. Priority targets of enzyme selection are antibiotics, hormones, and EDCs. Every step within the project is accompanied by in vitro biosensors for endocrine effects (estrogenicity, androgenicity and antiandrogenicity) to evaluate the degradation approach and assess potential new effects caused by generated transformation products. Degradation of target compounds was also monitored by on-line SPE-LC-MS-MS and data processed using TraceFinder 3.1. Several enzymes were discovered; and those ones from fungi (i.e., laccases from T. versicolor, M. thermophila) were the most promising, especially regarding EDC-degrading activity. The degradation of pharmaceuticals and hormones in real wastewater was already successful, such as tacrine and erythromycin which were degraded by 78% and 52%, respectively after 18 h. The next major aspect is a proof of concept, i.e. the feasibility of enzymatic treatment under “real world” conditions in wastewater. First experiments with endocrine active hospital wastewater showed a significant reduction of androgenicity and estrogenicity. For example the latter was reduced by 80% after 24 h. In addition to hormone, more work is needed to elucidate how mixtures of known endocrine disrupting chemicals were transformed in ultrapure water and in real wastewater. The degradation of EDCs with laccase is feasible, even at very low enzyme concentrations. The degradation by immobilized enzymes is faster compared to free enzyme but this tendency is superimposed by the adsorption of the carrier. Compared to the mixtures of known EDCs, we did not observe a degradation of unknown EDCs in wastewater this time. A solution would be a higher enzyme activity. These findings demonstrate that the enzymatic treatment technology can be an efficient and eco-friendly alternative or addition to existing technologies.

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Ozonation: a way to reduce antivirals in wastewater effluent? G. Fedorova, University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocosmides Vodnany Czech Republic; R. Grubic, University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocosmides; H. Söderström, Umea University

Ozonation is a well known water treatment method for the removal of heavy metals, microorganisms, and trace organic pollutants. In this study, we investigate the effect of ozonation on a range of antiviral substances.

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Organic micropolllutants (OMCs) in sewage treatment plants tend to sorb in suspended solids. Sewage sludge is mainly used as biosolid, consequently, to avoid the release of micropolllutants into agricultural land they might be removed during sludge anaerobic digestion (AD). AD is a complex process constituted by various steps that might have different influences on the removal of OMCs. Therefore, in order to increase the removal efficiency of these compounds, it is necessary to understand their behaviour and transformation. This study investigated the fate and biotransformation of usually detected micropolllutants in sewage sludge during methanogenesis, which is a key step in AD. To this end, kinetic experiments in a continuously stirred methanogenic digester were performed. The fate of an initial pulse of galaxolide (HHCB), toluamide (AHTN), ibuprofen (IBP), naproxen (NPX), sulfamethoxazole (SMX), trimethoprim (TMP), erythromycin (ERY), fluoxetine (FLX), and triclosan (TCS), estradiol (E2) and 17a-ethinylestradiol (EE2) was followed during time. The behaviour of the added OMCs was straight related to their solid–liquid partition coefficient ($K_L$); thus, some of them remained mainly in the liquid phase, meanwhile others sorbed into solids. In addition, experimental results suggest that biotransformation mechanisms could take place at different extents in the liquid and solid phase, depending on the OMC. Total concentrations were adjusted to a proposed biotransformation kinetic model, which seems to be appropriate to describe the behaviour of OMCs during methanogenesis and to quantify the capability of methanogenic biomass to biotransform OMCs. Several biotransformation kinetic models are being developed in order to simulate accurately the fate in both the liquid and solid phase of methanogenic sludge. In terms of removal, this study shows that methanogenesis has a marked impact on the low pressure lamp (PLP) degradation step in the biotransformation of SMX, TMP, E2, NPX, FLX and TCS. Accordingly, anaerobic digesters in sewage treatment plants should maximize the methanization of the feeding sludge in order the increase the removal of present OMCs.

515 Is combination of UVC and H2O2 effective to remove estrogens from water? B. CEDAT, COMAPINSA Lyon / LGCIE Water pollution is of major concern in France. Half of water resources was still not meeting the European quality requirement in 2012. Moreover, recent progresses in analytical chemistry have identified numerous micropolllutants in Wastewater Treatment Plant (WTTP) discharges. Fuels from urban, agricultural and industrial activities. Amongst hundreds of micropolllutants, estrogenic compounds were shown to cause endocrine disruption in fish at very low doses and suspected to impact human fertility. Therefore, UVC upgrade is needed to eliminate them. Advanced oxidation processes were shown to eliminate up to 80% of targeted micropolllutants. However small and medium sized WTP (< 10000 inh. eq.) are poorly equipped despite their representing 90% of the overall French WTP. Advanced Oxidation Processes (AOPs), based on the generation of highly reactive hydroxyl radicals for the unselective oxidation of chemical pollutants can be an adequate solution. This study aims at developing a cost-effective UV/H2O2 advance oxidation process (AOP) in order to reduce estrogenic activity in small and medium sized WTP discharge. Experiments were conducted at 20°C with a UVC reactor (50 W, UVC/UVB ratio of 15), and a 55% low pressure lamp (PLP) as key step in the biotransformation of SMX, TMP, EE2, NPX, FLX and TCS. Accordingly, advanced oxidation processes in sewage treatment plants should maximize the methanization of the feeding sludge in order the increase the removal of present OMCs.

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in winter 2013. Our part of the project focuses on assessing the overall health status of gammarids in the rivers Schussen and, in parallel, at the river Argen as a reference river. Two gammarid species were investigated in the present study: *Gammarus pulex* and *Gammarus roeseli*. Gammarids are excellent monitoring organisms, since they are widely distributed, occur in high abundances, are characterized by a rather short generation time, and are highly sensitive to pollutants. Samples were taken at different sites at the Schussen upstream and downstream of the WWTP, and in parallel, at the Argen river. Sampling took place several times prior and subsequent to the implementation of the activated carbon filter. Analyses of heat shock protein (hsp70) levels allowed us to draw conclusions about the general stress status of organisms based on proteotoxicity. Furthermore, the gammarid populations from all sites were investigated with respect to sex ratio, population density and species diversity. Based on the results of our study we can conclude that the gammarids’ health was impaired by the effluent of the WWTP Langwiese prior to the upgrading. Subsequent to its upgrade, however, organisms collected at the downstream site did not differ any more from gammarids collected upstream of the WWTP with respect to the investigated health parameters. Therefore, the efficiency of the activated carbon filter to eliminate toxic and endocrine chemicals from the effluent seems to be obvious. However, since results obtained subsequent to the upgrading are based on a dataset for a single year only, annual specific influences cannot be excluded. Further investigations are therefore required to strengthen the results.

517 Sediment ecotoxicity in Derian basins: toxicity bioassays and the Toxic Unit approach as complementary assessment tools

N. De Castro, Departament d’Ecologia; M. Kuzmanovic; N. Roig, Universitat Rovira i Virgili; J. Sierra, Universitat Rovira i Virgili / Soil Science Unit; A. Ginébre, CSIC - Spanish National Research Council / Department of Environmental Sciences; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research; M. López de Alda, Institute of Environmental Assessment and Water Research IDAEA-CSIC / Environmental Chemistry; S. Perez, IDAEA-CSIC / Environmental Chemistry; M. Petrovic, ICRA; Y. Pico, University of Valencia / Medicine Preventive; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering; I. Muñoz, University of Barcelona / Ecology

The quality of sediment is critical to the health of an aquatic ecosystem. Sediments can accumulate high diversity of pollutants, which can disrupt biological communities and alter the ecological status of ecosystems. Different bioassays have been developed in the last decades in order to evaluate the effects of the exposition of organisms to pollutants in sediment. These tests complement the information of the biological quality and the chemical characterization of the sediment carried out in situ. The main aim of this study was to determine the sediment ecotoxicity in four Iberian rivers based upon the results of chemical characterization, considering priority and emerging pollutants and biological responses. A battery of acute and chronic toxicity tests covering different trophic levels were conducted to evaluate the toxicity of pore water and whole sediment. The studied basins were the Ebro, the Llobregat, the Jucar and the Guadalquivir, and the test organisms were Vibrio fischeri, Pseudokirchneriella subcapitata, Daphnia magna and Chironomus riparius. Additionally, a broad spectrum of priority and emerging pollutants (EDCs, pesticides, PhACs, perfluorinated compounds, flame retardants, persistent organic pollutants and illicit drugs) were analysed in the sediment matrix. The Toxic Unit (TU) approach was applied to discern which compounds would explain the organism responses. EBR1 presented the highest pollution, followed by EBR2, LB8 and LLO5. Additionally, lead was found in EBR2, EBR3 and LLO5 for the *D. magna* test, and in JUC2 and JUC6 mortality levels were higher than 30%. The mortality of *C. riparius* was over 30% in EBR1, coinciding with the results found with *V. fischeri*. Lower growth rates respect to the control were detected in LLO5 (p<0.05). According to these results, the sediments that presented highest ecotoxicity were EBR1, EBR2, EBR3, GUA4, LLO5 and JUC2. The Llobregat river presented the highest Tu levels. GUA1 and EBR1 also presented high Tu levels. The main contribution to TUs were pesticides, mainly chlorpyrifos. Significant correlations were found between *C. riparius* endpoints and the *D. magna* TuS of PhACs, EDCs, illicit drugs and pesticides. These results confirm that the battery of toxicity tests together with the TU approach could be a good complementary tool for the determination of the ecological quality of these four studied river sediments.

518 The comparability of toxicity endpoints in bees

P.J. Holder, University of Exeter / Biosciences; C.R. Tyler, Biosciences College of Life Sciences; J.P. Campbell, Department of Environmental Science and Engineering

Convenient model species are regularly used in pesticide risk assessments to evaluate toxic effects for groups of species. For example, risk assessments for pesticide effects on pollinators are currently carried out mainly on honeybees. Many bee species however are exposed to pesticides applied to crops, with a wide range of negative effects, and there is doubt as to whether honeybees are representative of all bee species. Also, current risk assessments mainly focus on short-term, lethal endpoints. Here we used the response-response relationship to determine whether sensitivity to fipronil differs between two bee species (*Bombus terrestris* and *Apis mellifera*) and several endpoints for toxicity testing. We plotted the responses of bees exposed to identical doses of fipronil under laboratory conditions against one another to determine whether different species and endpoints are equally sensitive. Bumblebees were more sensitive than honeybees to dietary fipronil, up to 125 µg L⁻¹, for the endpoints of longevity and feeding. Fecundity was found to be more sensitive than both mortality and pollen feeding as an endpoint of bumblebees exposed to dietary fipronil, up to 1.28 µg L⁻¹, within queenless microcolonies. In conclusion, the bumblebee (*Bombus terrestris*) would make a better model species than the honeybee (*Apis mellifera*) for determining toxic effects on a social pollinator due to its increased sensitivity to toxicants across lethal and sublethal endpoints. Among the endpoints we tested, bumblebee fecundity would make a suitable endpoint for regulatory testing due to its higher sensitivity to toxicants. Fecundity is an ecologically realistic endpoint which is reduced at field realistic concentrations of fipronil and is demographically relevant to protection goals.

519 Identification of trans- and cis-eQTLs in Lead-treated Drosophila Model

W. Qu, Wayne State University / Pharmacology; R. Pique-Regi, Wayne State University / Center for Molecular Medicine and Genetics; D. Ruden, Wayne State University / Department of OB/Gyn

Lead poisoning has long been a major public health issue globally. In order to better understand how lead plays a role as a neurotoxin, we utilized the *Drosophila melanogaster* model to study the genetic effects of lead exposure during development and identify the lead-sensitive genes. Our group utilized the eight *Drosophila* recombinant inbred lines (RIls) from the *Drosophila Synthetic Population Project*. RNAseq was conducted and expression quantitative trait loci (eQTL) analyses were validated using both trans-eQTLs using microarrays (Ruden et al., 2009). What we have found is that approximately 2/3 of total cis-eQTLs were shared in both control and Pb-treated data. However, many of the trans-eQTLs found in control samples shifted their positions after treatment with lead. cis-eQTLs are regulatory genetic sites, whereas trans-eQTLs are regulators that are not genetically linked to the gene. The shifting of entire trans-eQTL networks in the presence of lead was an unexpected finding in our original study that we replicated in the current study. Using RNA-seq analysis also helped narrow down each trans-eQTL network from the previous 5Mb to within 1Mb, which makes future experimental validation and the identification of causative single nucleotide polymorphisms (SNPs) in the trans-eQTL possible. A future advantage of RNA-seq over microarrays is that we can also identify splicing QTLs (sQTLs), which are SNPs that regulate alternative mRNA splicing. The identification and validation of both trans-eQTLs and sQTLs will be the basis of our future research on this project.

520 Ecotoxicological effects of post-fire runoffs on aquatic invertebrate detritivores

F.A. Carvalho, University of Minho / CBMA Center of Molecular and Environmental Biology Department of Minho University / Center of Molecular and Environmental Biology (CBMA), Department of Biology; N. Abrantes, University of Aveiro / CESAMDAO; C. Paçoal, F. Cassio, University of Minho / Centre of Molecular and Environmental Biology (CBMA), Department of Biology

Mediterranean forests are highly susceptible to wildfires, which can cause several impacts not only on burnt areas, but also on adjacent ecosystems. The washout of heavy metals from forest burning areas may trigger an increase in eutrophication of freshwater bodies, with the potential for toxic effects on aquatic organisms. However, there is still a lack of knowledge on the impacts of post-fire runoff on stream bio. Here, we used aqueous extracts from wildfires obtained in different areas (pine trees, high severity; eucalypt trees, high severity and a stream) in Macida (Sever do Vouga, Portugal) to evaluate their effects on the feeding behavior and biochemical responses of the invertebrate detritivore *Allogromas spurus*. The feeding activity was assessed by exposing animals for 20 days to increasing concentrations (0, 50, 75 and 100%) of aqueous extracts and allowing them to feed on leaves previously colonized in a stream by microorganisms. To assess the biochemical responses, we measured the oxidative stress biomarkers glutathione peroxidase (GPx), glutathione S-transferase, and the neuronal stress biomarker cholinesterases, in invertebrates exposed for 96 hours to increasing concentrations of the aqueous extracts. The invertebrate feeding rate decreased to 10% comparing to control when animals were exposed to the highest extract concentration of wildfire aqueous extracts from the eucalypt plantation. Severe inhibition of the feeding rate was also found (feeding exposure to eucalypt fire extracts decreased to 18% at 75% extract concentration). Stream extracts had the least severe effects on the invertebrate feeding rate, which was reduced to 50% at the highest concentration. After exposure to the aqueous extracts, the activity of oxidative stress enzymes increased significantly in a concentration-dependent manner, while cholinesterase activity decreased. Overall, results suggested that post-fire runoff affects the behavior and the physiology of invertebrate detritivores with potential impacts to their ecological role in streams. Acknowledgements: FEDER-POFC-COMPETE and FCT supported this work (PIDDAC - PEst-OE/BIA/UI4050/2014, PTDC/AAG-GLO/4176/2012)
Multistress in aquatic environments: the big picture (III)

522 Influence of temperature on the tolerance capacities of phototrophic and heterotrophic biofilm communities to copper
A. Lambert, Irstea Lyon; A. Dabrín, Irstea centre de Lyon Villeurbanne; UR MALY; S. Morin, Irstea / UR EABX; J. Gahou, Irstea Lyon; S. Pesce, Irstea / UR MALY

Agricultural rivers are highly affected by metal pollution, with copper (Cu) being frequently detected and impacting aquatic organisms. In lotic ecosystems, periphytic biofilms assume key ecological functions such as primary production and nutrient cycling. These microbial assemblages are formed by phototrophic and heterotrophic communities which are the first to interact with dissolved substances, including Cu. Chronic exposure of biofilms to Cu can induce a succession of phototrophic and heterotrophic species resulting in a replacement of the sensitive species by more tolerant ones, thus leading to an increase in the tolerance of the microbial communities to this toxicant (concept of pollution-induced community tolerance, PICT). However, the pollution of surface waters and the resulting ecotoxicological effects must be viewed in a context of global change since aquatic systems are generally subjected to multi-stress conditions. Given the increase in extreme climatic events due to climate change, acute variations in temperature may represent an environmental stressor influencing microbial communities. Indeed, even if effects of Cu on microbial communities are relatively well known, there is a lack of data about the possible influence of temperature on the tolerance capacities of biofilm to copper. Accordingly, the present study aimed to evaluate in microcosms the influence of temperature on the tolerance level of microbial biofilm communities to Cu. Natural summer communities, collected in a 18°C stream water, were subject to temperature shocks to 3 thermal conditions (18°C, 23°C and 28°C) in presence or not of Cu. Tolerance levels of phototrophic and heterotrophic communities were evaluated using short-term toxicity tests targeting photosynthetic (yield) and enzymatic activities (β-glucosidase and leucine-aminopeptidase), respectively. At the initial river temperature (18°C), our results confirmed the tolerance increase to Cu after chronic exposure of both phototrophic and heterotrophic communities. However, the increase in temperature only had a limited effect on the tolerance level of communities pre-exposed to Cu. Our results showed a great influence of temperature on the Cu tolerance level of both phototrophic and heterotrophic communities that were not pre-exposed to Cu. It reveals that temperature is a primordial factor to take into account in the assessment of tolerance capacities of biofilms to metal stress, especially when using PICT approaches.

523 Salinisation effects on coastal freshwater ecosystems due to climate changes
C. Venâncio, Biology; B.B. Castro, N. Abrantes, University of Aveiro / CESAMDAO; E. Anselmo, CESAM University of Aveiro; S. Antunes, University of Oporto / Biology; M. Moreira-Santos, University of Coimbra, IMAR–CMA / Department of Life Sciences; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; R. Ribeiro, University of Coimbra; I. Lopes, University of Aveiro / CESAM Biology Department

Recent projections of the International Panel for Climate Change comprise among others awareness regarding global sea level rise. Though some knowledge exists on the impacts of salinity on freshwater ecosystems, a considerable uncertainty on salinisation effects to the ecosystems still remains, denoting the need for further detailed investigations on this issue. This work intended to contribute for a better prediction of the effects provoked by salinisation to freshwater coastal ecosystems. For this, four specific objectives were delineated: (i) determine the ecological receptors at most risk, (ii) determine the ability of freshwater species to acclimate to salinity, (iii) determine if an association exists between tolerance to salinity and other chemicals, and (iv) assess the influence of salinity in interspecific relationships (competition). To attain specific objective (i) and (ii), lethal and sublethal monospecific assays were performed by exposing species, from different trophic levels, to increased salinities (due to natural seawater and NaCl) before and after being acclimated to low levels of salinity. To address specific objective (iii) six clonal lineages of Daphnia longispina were exposed to lethal levels of increased salinity and of four metals (copper, zinc, cobalt, chromium), to study the associations between the sensitivity to salinity and metals. To tackle specific objective (iv) microcosms experiments were done with two species of microalgae exposed to increased salinity scenarios under the absence and presence of inter-species competition. Obtained results suggest that, in a test-screening phase, NaCl was applied as diagnostic tool to detect effects conferring on a former effect-directed analysis (EDA) study on sediments, which revealed three phytotoxic compounds in a multiple contaminated site. In a case study, the impact of three site-specific, phytotoxic compounds on periphyton was investigated in situ. Periphyton was cultivated at a reference (R-site), previously analysed by EDA. The sensitivity of R- and P-site communities to prometryn, tributyltin and N,N-di(2-naphthyl)ethylenediamine was determined by exposure to lethal and non-lethal levels of increased salinity. Our pioneering work provides a foundation for automated high throughput ecotoxicity biotests using innovative Lab-on-a-Chip technologies. We are convinced that microfluidic technologies can provide new vistas for high-throughput ecotoxicology studies on selected aquatic test species. A key advantage of microfluidic systems as compared to conventional static “Petri dish” testing is the miniaturization and automation of analysis and special emphasis on analysis performed under continuous micropuarfusin. We anticipate that rapid development, validation and adaption of miniaturized fluidic technologies will bring us a step closer to realization of full laboratory automation in ecotoxicology
abundance was measured using epifluorescence microscopy (DAPI-staining) and bacterial production was quantified with 14C-leucine incorporation techniques. Bacterial community diversity was identified using DGGE on rpoB gene amplicons. Acidification alone consistently reduced bacterial abundances and stimulated production. A clear combined stress effect of pH and antibiotics was observed acting on all measured variables, where the presence of Amox augmented the pH effect while Cip hampered or even reversed it. The less diverse winter bacterial community appeared more sensitive and thus less resilient compared to the more diverse spring bloom community. As predicted, the bacterialic effect of Amox was greater at low pH while the opposite was true for Cip. We conclude that impact assessments of ocean acidification on marine organisms should include effects of pH induced change in polar contaminant bioavailability and toxicity.

### Soil Ecology and Ecotoxicology: Pillars Supporting the Conservation and Protection of Soil Biodiversity and Ecosystem Services during the International Year of Soils

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**Soil bioindicators: hSoIL properties influence their responses and how to select them in function of the site issues?**

B. Pauget, University of Franche-Comté, UMR UFR CNRS 6249; L. Rougé, AgroParisTech/ADERA; A. Huttin, IUT Clermont Ferrand; N. Laurent, C. Villenave, Université de Franche-Comté / UTC ChronoEnvironment; J. Cortet, Université de Montpellier; S. Croquet, Université Cézanne; S. Dequiedt, INRA Dijon; O. Faure, Ecole Nationale Supérieure des Mines de Saint-Etienne Centre SPIN / CNRS / UMR 5600 Environnement-Ville-Société; C. Gangneux, I. Gattin, Estipa; M. Le Guédard, LIEC CNRS / Université de Lorraine; C. Pouget, Université de Caen; J. Ribot, IRD Bondy; S. Taibi, Estipa; F. Vidal, C. Vidal-Ulcke, LIEC CNRS / Université de Lorraine; C. Vidal-Esteve, Unile Ville Fonctionnement des Ecosystèmes Terrestres Anthropisés Cité Scientifique SF Rde VF Villeneuve d'Ascq France; A. de Vaulx, University of Franche-Comté / Department of ChronoEnvironment; C. Villenave, ELISOL Environnement; G. Péris, INRA Agrocampus Introduction – Despite no European directive exists for soil protection, this key component of ecosystem services needs to be protected. To fill the lack of tools to monitor the soil threats and to assess the impact of soil management, biological indicators have been developed in the French program Bioindicators 2 (ADEME). To facilitate the choice of relevant bioindicators by end-user depending on their problematic, the objective was also to develop a web interface. On the 47 plots of 13 sites (agricultural, industrial and forest), the influence of soil characteristics and soil use on the response of 80 biological parameters (fauna, flora and microorganisms) have been assessed by using linear multivariate regressions. Results - The soil texture and the organic carbon rate have been identified as the main soil characteristic influencing the biological responses, even if each bioindicator is influenced by its own pool of soil parameters. It underlines the necessity to use balanced bioindicators assessment for soil quality and other environmental purpose. In other words, the use of soil contaminants (organic or metallic) have been highlighted for 86% of the bioindicators showing the necessity to consider their bioavailability for suitable soil management. A web interface has been implemented (http://ecobiosoil.univ-rennes1.fr/ADEME-Bioindicator/). It presents all the biological methods developed in the program and allows consultation of the variation range of each bioindicator considering site context and soil physico-chemical characteristics of soils thus leading to provide a first benchmark. It also provides tools to the end-users to select the bioindicator(s) which best fit(s) with their own site problematic (agricultural practices, bioavailability of contaminants, soil monitoring...) and to communicate (technical sheets...). Conclusion – For the first time, the soil properties influence on the response of 80 bioindicators was characterized under similar environmental conditions. As each bioindicator is influenced by a specific pool of soil parameter, chemical measures cannot be used as a surrogate of biological measures. This program has provided usable tools for a biologically-based site management.

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**Incorporation of a new A horizon 'soil fingerprinting' framework into soil ecotoxicity assessments**

N.C. Feisthauer, C.A. Fox, Agriculture and Agri-Food Canada / SCIENCE AND TECHNOLOGY BRANCH; G. Broll, University of Osnabrueck / Institute of Geography; C. Tamanoi, E. Kenney, D. Kroetsch, Agriculture and Agri-Food Canada / SCIENCE AND TECHNOLOGY BRANCH; T. Rosschau, Lethbridge Centre for Agricultural Landscape Research / Institute for Landscape Biogeochecmistry Soil quality is fundamental to soil biodiversity, function and the provision of services such as food production, and is derived from characteristics of soil biological, chemical and physical components. Each of these components influences the response of soil organisms to contamination and should be considered in the interpretation of ecotoxicity assessments. However it is difficult to describe the constituents of soil physical, chemical and biological components in...
a manner that is succinct, repeatable and has the potential to be quantified. A unique "soil fingerprint" framework was developed to record and monitor the impacts on soil quality from environmental stressors and land use management. The framework provides an enhanced soil horizon description methodology to systematically track and record change in soil chemical, physical and biological characteristics. The enhanced horizon description generated is analogous to a genetic fingerprint for an individual soil sample at the time the sample was collected. The framework monitors horizon characteristics that do not only represent dynamic soil properties (e.g., soil structure, organic carbon, etc.) but also soil and land information (e.g., soil texture, land use) providing data to allow comparative interpretations of soil quality changes and identify trends due to human activities (e.g., soil management practices, remediation, etc.) among diverse soils or the same soil under different conditions. The design of the framework is modular, meaning any level of information can be removed and new levels can be added. For example, levels for soil microbial respiration, root biomass, microarthropod toxicity etc. can be added.

The framework then generates fingerprints that are unique identifiers of the nature and context of the key dynamic soil properties related to soil quality as one integrated descriptive label together with ecotoxicity information. For instance, by including soil structure, the framework can integrate physical characteristics that significantly influence soil biological endpoints but that are not often included in ecotoxicity assessments. A database of fingerprints, generated either over time or among sites, provides enhanced opportunities to quantitatively analyse multiple lines of evidence in contaminated site assessments.

530 Soil microbial response to toxicants varies with soil moisture and temperature S. Thiele-Bruhn, University of Trier / Soil Science; R. Reichel, Department of Soil Science

In general, it is not regulated or considered that the status of soil biota is not constant but may vary depending on soil moisture and temperature. This might influence the biodiversity of soil organisms as well as their susceptibility towards chemicals. The effect of different soil moisture and soil temperature on the susceptibility of soil microorganisms towards selected toxicants was tested. To this end, soils were either pre-incubated at three different temperatures (10, 20, 30 °C) or (b) at three different soil moisture levels (30, 50, 70% WHCmax). Microbial respiration, microbial biomass Cmic and Nmic and dehydrogenase activity were determined as endpoints to investigate dose-related effects of bronopol. In a second step, soil samples were exposed to repeated drying and rewetting cycles in a 49-d fully controlled climate chamber pot experiment with planted soil. Sulfadiazine was added with manure and effects on microbial community structure were determined from fructose-1,6-diphosphate after 1 and 7 days of incubation. A stronger effect on samples with higher biomass and respiration activity in the control corresponds with the biostatic mode-of-action of the pollutant that requires an active microbial community to exert its effect. Drying-rewetting influenced the microbial community. Significantly lower total PLFA concentrations (p < 0.05) were determined in sulfadiazine treated samples than when they were additionally exposed to drying-rewetting cycles. Similar results were found by 16S rRNA gene analysis. Significant shifts (p < 0.05) in the B- proteobacteria and pseudomonas community structure were only not on drying-rewetting but even more on the direction of the soil moisture change. It is concluded that toxicity tests with soils not only require the definition and standardization of test parameters but also of the pre-treatment of the soil samples. Also, it must be expected that effects of pollutants in a field soil may significantly deviate from the results of laboratory tests.

531 Spatial and temporal dependency of ecosystem services on soil processes in invaded grasslands. S. D. Siciliano, University of Saskatchewan / Toxicology Group and Department of Soil Science; E. Lamb, Saskatchewan University / Plant Ecology and Biostatistics Department of Biology, University of Saskatchewan

The protection of soil processes from abiotic and biotic stressors is rationalized on the basis that these processes are linked to critical ecosystem services. Ecosystem services such as climate regulation, pollutant degradation, nutrient cycling, biomass degradation and forage quality are spatially dependent and temporally variable across a landscape. Thus, in order to link soil processes and community structure to ecosystem services, one should incorporate spatial and temporal variability of these services and their corresponding link to the soil community. We investigated the temporal and spatial dependency of ecosystem services and soil community structure across a in a rangeland undergoing smooth brome invasion from soil thaw to freeze (26 weeks) in the summer of 2014. Smooth brome invaded soil samples had substantially altered carbon dioxide flux, nutrient cycling and biomass recycling compared to the native plant community. Invaded soil communities had increased C, N and S cycles but had decreased biomass decomposition activity. Other indicators of soil community structure such as dehydrogenase or phosphatase activity were not influenced by invasion and gross physical parameters such as moisture and temperature were also not affected by invasion. In previous work we demonstrated that there were substantial changes in microbial richness and nitrogen cycling associated with smooth brome invasion. If this trend continues at this site, it appears that these alterations in microbial community structure result in changes in ecosystem services.

532 Wastes or products? Can agricultural application be (part of) the solution for the disposal of organic wastes? J. Renaud, Department of Life Sciences; S. Chelinho, IMAR - CMA / Department of Life Sciences; P. Alvarenga, Polytechnic Institute of Beja / Department of Technologies and Applied Sciences; C. Mourinha, Polytechnic Institute of Beja; P. Palma, Instituto Politécnico de Beja / Department of Technologies and Applied Sciences; I.M. Sousa, Faculty of Engineering of University of Porto / Chemical Engineering; T. Nata de Luz, University of Coimbra / Department of Life Sciences In recent years the production of organic wastes has increased rapidly and their deposition in landfills constitutes an unsustainable way of waste disposal. Thus alternatives must be found. The use of organic wastes as soil amendments in agriculture could be an important alternative benefiting from their high nutrient and organic matter content and also their positive effects on the aggregation and structural stability of soils. However, this practice should be carefully monitored for the environmental risk posed by many wastes due to the presence of substances with hazardous effects on soil organisms. So, how to properly assess the risks of wastes? With this question in mind, and based on data from reproduction (Enchytraeus crypticus, Hypoaspis aculeifer, Eisenia fetida) and germination tests (Lactuca sativa and Lolium multiflorum) for 9 different organic wastes, this presentation aims at discussing the challenges and necessary changes to implement in a foreseeable assessment scheme to better unravel the potential risks of organic wastes and their use as soil amendment products. Results obtained demonstrate that organism responses were not consistent and no general sensitivity trend across all wastes could be found. This supports the need for a test battery including several organisms to account for differences in sensitivity and in exposure routes to adequately evaluate environmental impacts. Furthermore, wastes with low contaminant loading in some cases showed higher toxicity than wastes with high levels of contaminants. This suggests that chemical analysis per se is not sufficient to assess environmental risks since it may over or under estimate the toxicity of wastes. This also indicates that the list of parameters for analysis should be extended beyond traditional contaminants (e.g., pH and salinity) that, in some cases, may be the ones originating toxicity. Overall the re-use of wastes as soil amendments would benefit the management of waste disposal and improve soil quality for agricultural crops reducing the need of inorganic fertilizer application. This strategy could also aid in promoting good practices for waste producers in order to reduce potential hazards and be eligible for agricultural application. This study highlights the need to expand the list of chemical parameters to screen and the need of including ecotoxicological tests as a complement of chemical analysis to evaluate the quality of wastes as soil amendments.

533 Novel endpoints for the Enchytraeid Reproduction Test - hatching, growth, full life cycle - to estimate population dynamics R.C. Bicho, Universidade de Aveiro / CESAM Department of Biology; F.C. Santos, Biology CESAM; M. Gonçalves, University of Aveiro, Department of Biology & CESAM / Biology; A.M. Soares, A.M. Amorim, Universidade de Aveiro / Department of Biology and CESAM

Soil toxicity standard tests for soil pollutates are usually limited to the assessment of endpoints like survival and reproduction. Adverse effects may occur at other developmental stages, e.g. embryo development, hatching or growth. The species Enchytraeus crypticus is a model organism in the standard soil ecotoxicity test, where survival and reproduction are assessed. In the present study we optimized the method to include additional endpoints. Tests start with synchronized age organisms', a major advantage compared to the standard. The test includes additionally hatching success, growth, maturation status and full life cycle. Moreover, cocoon production and population growth rate were calculated. Cadmium was used as validating test substance. Results indicated that Cd is embryotoxic, main effect occurs on the embryo developmental stage and maturity. Further, the full life cycle test can discriminate between pre- and post-embryo formation and provides life-history information that can be integrated to predict population increase and population growth rate.

Innovations in environmental analytical chemistry: the quest for pollutants using target and non-target approaches (III)

534 'Semi-Targeted' Analysis of the River Thames: Combining Targeted and Non-Targeted Approaches for Pharmaceutical and Illicit Drug Residue Detection K. Mugan, Analytical and Environmental Science; A. Edge, C.P. Martins, Thermo Fisher Scientific; D.A. Cowan, Kings College London / Drug Control Centre; L. Barron, Kings College London / Analytical and Environmental Science

The potential of suspect/non-targeted analytical approaches using high resolution analytical techniques for pharmaceuticals and related contaminant identification in...
the environment has been widely acknowledged in recent years. By developing better post-data acquisition mining tools, a larger number of unknown contaminants can be potentially identified in parallel with a traditional targeted analysis. A more comprehensive understanding of the true breadth of pharmaceutical occurrence in the environment can therefore be gained. Presented herein is the development of a ‘semi-targeted’ screening method using solid phase extraction (SPE) and liquid chromatography-high resolution mass spectrometry (LC-HRMS) for a temporal and spatial study of drug occurrence in the River Thames. This novel ‘semi-targeted’ approach combines targeted analysis, through determination of pre-selected analytes, with non-targeted analysis, allowing retrospective identification of unknowns using optimised data-mining screening tools. A broad SPE extraction method was developed using 45 structurally diverse compounds. Development was pursued in a High Resolution Mass Spectrometry (HRMS) to achieve a comprehensive quantification of target analytes and qualitative semi-targeted analysis for additional pharmaceutical content. Data-mining software was used to develop and apply an optimised screening method, using matrix-matched reference standards to confirm the presence of unknown contaminants. Grab samples were collected during two week-long river sampling campaigns, at two sites covering a tidal region of the Thames which receives raw untreated wastewater from 7 combined sewer overflow (CSO) vents during times of heavy rainfall. In general, no overall temporal or spatial trends were observed, with any variation remaining on a compound specific basis. An apparent spike in concentration of caffeine was observed, which could be indicative of a CSO event, and will be discussed in combination with rainfall data. The optimised data-mining screening method was used to have a high throughput of the metabolites in the river water samples. The presence of 12 shortlisted unknown suspects was confirmed using a matrix-matched standard. Overall, by combining a targeted quantification method with a qualitative non-target approach, the applied semi-targeted method resulted in a more comprehensive analysis of the river, underlying its potential in the analysis of pharmaceuticals in the environment.

535 Suspect screening of pharmaceuticals and their metabolites in fish by UPLC-HRMS. J. Aceña, I.DAEA-CSIC; L. Benejam, University of Girona / Institute of Aquatic Ecosystems; S. Perez, UB-SEAWATCH / Environmental Chemistry; D. Barberà, IQAB-CSIC / Institute for Environmental Assessment and Water Research In the last decade, the occurrence of pharmaceuticals in rivers has caused concerns about potential adverse effects on exposed wildlife. Although some studies have been reported the occurrence of pharmaceuticals in different fish tissues, still very little is known about their disposition in fish. In this context, we wanted to evaluate the occurrence of suspected drugs and their metabolites in the bile of fish. To this aim, we performed a preliminary study identifying bile metabolites in fish placed in tanks and treated with two of the most commonly detected pharmaceuticals, carbamazepine and ibuprofen. Once the identification of fish metabolites was accomplished, fish were collected from rivers, their bile isolated and analyzed for the identified metabolites and the corresponding parent drugs. In order to have a high throughput of the metabolites in the river water samples. Those pharmaceuticals which are found in river waters, a suspect screening of drugs and their predicted metabolites in fish bile and muscle was evaluated by ultra-high performance liquid chromatography - high resolution mass spectrometry (UPLC-HRMS). The accurate mass measurements obtained by HRMS allowed screening for suspected drugs and their metabolites and provided plausible chemical formulae. The MS/MS spectra of metabolites and the parent compounds allowed to propose plausible structures for metabolites in fish bile. With this approach phase I and phase II metabolites were identified. The suspect analysis of muscle allowed the detection of more than ten different drugs and demonstrated the uptake of drugs from water and their plausible accumulation. Psycho-active drugs were one of the most commonly detected drugs. Their identities were proposed by matching their accurate MS and MS/MS data against different libraries. Finally, authentic standards were employed to confirm the proposed drug identities and to determine analyte concentrations in the fish samples. Concentrations of pharmaceuticals were determined in the range of ng/g. This approach highlights that UPLC-HRMS is a powerful tool for simultaneous quantitative and qualitative analysis, allowing the suspect identification of compounds, their identification and the quantification of target compounds. Furthermore, UPLC-HRMS provides a great deal of information about each sample, allowing the retrospective analysis.

536 Detection and identification of new psychoactive substances in pooled urine using High Resolution All-Ions MS/MS. J. Kinyanj, University of Antwerp / Pharmaceutical Sciences; N. Negreira, University of Antwerp / Faculty of Pharmaceutical Sciences; A.A. van Nuijs, University of Antwerp; M. Ibanez, DELTARES / Marine and Coastal Systems; L. Bijsma; M. Biseraz, University of London; F. Hernandez, Universitat Jaume I; A. Covaci, University of Antwerp, Toxicological Center / Toxicological Centre New Psychoactive Substances (NPS) are compounds that mimic effects of illicit drugs like cocaine, cannabis and amphetamines— and are synthesized to evade law enforcement by introducing slight modifications to chemical structures of controlled substances. Currently, there are around 330 NPS in the market, most of which have no commercially available reference standards making it challenging to develop targeted methods for their analysis. In addition, their use is very sporadic depending on popularity amongst users. High-resolution mass spectrometry (HRMS) using quadrupole time-of-flight (QTOF) analyzer is a useful technique for wide-scope screening since it provides sensitive, full-spectrum MS data with high mass resolution and accuracy. All Ions MS/MS (also known as MSE) is a technique that is available for liquid chromatography-HRMS (LC-HRMS) and is used for providing targeted screening with the advantage of using these data in the future, in a retrospective analysis searching for additional compounds without the need of re-injecting the sample extracts.

537 UHPLC-HRMS characterisation and toxicity assessment of ofloxacin transformation products after TiO2 nanoparticles and TiO2 nanofibers photocatalysis. J.J. Villarr, Analytical Chemistry; L. Meschede Anglada, A. Sierra Chussellas, A. Conesa Baeza, Letatit Technological Center; E. Moyano Morcillo, University of Barcelona. 1. Introduction There is an increasing concern over antibiotics and their presence in the environment, since they and/or their metabolites could induce bacterial resistance. Amongst antibiotics, fluoroquinolones are a class of antibacterial agents widely used for human and veterinary applications and conventional sewage treatment plants are not able to efficiently remove these chemicals. In this study, the degradation of the fluoroquinolone ofloxacin (OFX) was under investigation using heterogenous photocatalysis based on TiO2 nanoparticles (NPs) and nanofibers (NFs). Effect directed analysis (EDA) was used to study the potential toxicological activity of the generated transformation products (TPs) during the photocatalytical process. 2. Materials and methods Photocatalytic experiments with suspended and supported TiO2 NPs or NFs were performed in a borosilicate photochemical reactor. Eight HPLC fractions were collected and bio-tested using vibrio fischeri and HepG2 and HK2 human cell lines. In silico predictions were performed with the OECD QSAR Toolbox v3.2 and T.E.S.T software. 3. Results and discussion Suspected TiO2 NPs achieved complete OFX removal faster than TiO2 NFs. No significant differences were observed between TiO2 NPs and TiO2 NFs under supported conditions, achieving complete removal in 240 minutes. Having identified 60 OFX TPs, six main routes were established to be promoting its degradation. Some of these TPs were found to be persistent, as no complete removal was achieved. In-vitro studies revealed that alliquotes collected at 15 and 20 minutes showed presence of processive effects. Tentative tentatives (28%) were correlated to identify the compounds after fragment elucidation. This strategy is a useful tool in target and suspect screening with the advantage of using these data in the future, in a retrospective analysis searching for additional compounds without the need of re-injecting the sample extracts.

538 Comprehensive two-dimensional liquid chromatography coupled to time of flight mass spectrometry for effect-directed analysis of wastewater treatment plant effluent in the Netherlands. X. Ou Yang, Institute for Environmental Studies IVM; J. Legradi, Institute for Environmental Studies; P. Leonards, VU University, Institute for Environmental Studies / Institute for Environmental Studies IVM; J. de Boer, VU University / IVM; R. Van der Oost, Wateren; J. Legler, VU University / Institute for Environmental Studies; L. Lamoree, VU University, Institute for Environmental Studies / Institute for Environmental Studies Due to massive human activities such as agriculture, wastewater discharges and industrial manufacturing, potentially harmful chemicals reach the environment. These chemicals are large in number and possess diverse physicochemical properties, ranging from non- or weakly polar compounds such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) to strongly polar compounds such as novel pesticides, pharmaceuticals and personal care products
Comprehensive two-dimensional liquid chromatography (LC×LC) is an emerging technique that has been applied in areas of notable sample complexity, due to its greater peak capacity and tunable selectivity by different stationary phase combinations. In our study (EDA-EMERGE project, EU contract 290100), for the first time a LC×LC system linked to a 96-well plate collector, a UV detector and a high resolution time of flight mass spectrometer (ToF-MS) was developed to support the analysis of emerging contaminants in Effect-Directed Analysis (EDA). The 96-well plate format (project sponsored by the BE-BBB Foundation) was fractionated into 4×96 well plates and the collected fractions were tested for developmental toxicity using a high throughput zebrafish embryo toxicity (ZFET) assay and also an acetylcholinesterase (AChE) assay. The performance of an LC×LC system was explored with regard to its potential for the analysis of standard mixtures of environmental contaminants. In LC×LC experiments we have demonstrated that a significant enhancement of the peak capacity facilitates the rapid identification of toxic compounds by online LC×LC-ToF MS and high throughput toxicity testing, leading to a comprehensive chemical characterization of environmental water samples, including toxicological evaluation of the identified compounds.

539 Linking Influent and Effluent Peaks from Biological Wastewater Treatment to Detect Relevant Nontarget Compounds

J. Scholle, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Chemistry; E. Schymanski, Eawag Swiss Federal Institute of Aquatic Science; S. Avak, Eawag Swiss Federal Institute of Aquatic Science and Technology; J. Hollender, Eawag / Environmental Chemistry

Transformation products (TPs) are formed during the wastewater treatment process (WWTP) and lab studies and targeted screening can only provide a limited picture of the compounds present. This project focuses on using nontarget screening with liquid chromatography coupled to high-resolution mass spectrometry (LC-MS/MS) and tandem mass spectrometry to develop new compounds as micropolutants. With a combination of LC-HRMS, MS/MS, and multivariate analysis, prioritization of the nontarget peaks was done to select compounds interesting for structure elucidation. Samples were enriched with solid-phase extraction and were analyzed by LC-HRMS using electrospray ionization in positive and negative mode. Pre-processing was carried out with the software R, including peak picking, isotope/adduct clustering, and feature building. Principal component analysis (PCA) was used to classify peaks based on where they are most often detected (i.e., in the influent or effluent samples). Links between the two groups were explored based on known biotransformation reactions. When matches were found, prioritization was done using peak shape and intensity, significance of the intensity difference between the two sample types, and similarity of the MS/MS spectra. From the PCA, 9738 features were classified as potential parents and 3011 features as potential TPs. Using 7 biotransformation reactions, 4249 potential links were found. From this, 156 were selected for targeted MS/MS based on the workflow prioritization. MS/MS fragmentation from the proposed parent and TP were compared, and 19 links with an MS/MS similarity score of higher than 0.5 were selected for further structure elucidation. In one example, the MS/MS fragmentation of the parent and TP were similar; both contain fragments at masses 103.0390 and 89.0597. Other linkages were also found with these fragments. The mass difference between the two parent compounds was 44.0264 mDa [C₂H₅O]. As homologue series such as this have been reported previously in WW, this series was extrapolated to higher and lower masses and the EICs visualized. After the treatment, some lower masses were removed, although some of the higher mass homologues appear unchanged or increased after the treatment. Preliminary results indicate that by applying multivariate analysis and a nontargeted workflow, relevant compounds can be found using these methods, not only between parent and TP but also potentially new homologue series.

Recent scientific developments in bioaccumulation research and assessment

540 Bioaccumulation Assessment: Developing Frameworks and Finding Common Ground

I.A. Arnott, ARC Arnott Research & Consulting / Department of Physical Environmental Science; J.W. Nichols, U.S. EPA / ORD NHEERL; Mid Continent Ecology Division; M. MacLeod, ITM - Stockholm University / Dept of Environmental Science and Analytical Chemistry ACES; F. Papa, Department of Theoretical and Applied Sciences; K. Borgia, Department of Biosciences, University of Oslo / Department of Biosciences; H. Lau, Givaudan Schweiz AG / Fragrances S T; P. Leonards, VU University, Institute for Environmental Studies / Institute for Environmental Studies IVM; F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management Faculty of Environment

Bioaccumulation is the net result of competing rates of chemical uptake and elimination in an organism. Various methods, metrics and criteria have been developed to describe and predict bioaccumulation (B) assessment. The octanol-water partition coefficient (Kow) and the bioconcentration factor (BCF) are B metrics currently implemented in regulatory programs. Alternative metrics for B assessment have been proposed including the biomagnification factor (BMF), the trophic magnification factor (TMF), the bioaccumulation factor (BAF), and the total elimination half-life (HLₐ). Mass balance models and quantitative structure-activity relationships (QSRs) are often useful, particularly for screening-level assessments. A Bioaccumulation Workshop organised and sponsored by the European Chemical Industry Council-Long Range Research Initiative (Cefic-LRI) was hosted at the European Chemicals Agency (ECHA) in Helsinki, Finland. This presentation proposes a path forward for improving bioaccumulation science and assessment. All data (measured or predicted) need to be critically evaluated and carefully interpreted for relevance and reliability. Mass balance (process) models provide a framework to incorporate and compare various sources of bioaccumulation information (in vivo, in vitro, in silico, in situ) for a range of bioaccumulation assessment endpoints of interest. The framework can be used to determine if there is consistency or divergence in theoretical and empirical knowledge, thus providing confidence in B assessment. Definitive B evaluations should be based on a weight-of-evidence approach. A common thread for B assessment and a key model input parameter for the reliable prediction of BCF, BAF, BAF, TMF and HLₐ endpoints for hydrophobic chemicals in fish and other species is the primary biotransformation rate constant kₜ (or kₐ). In vitro, in vivo and in silico methods for estimating kₜ have been developed and evaluated. Confidence in kₜ can be obtained by corroborating in vivo, in vitro and in silico estimates and the applicability domains of current HLₐ-QSRs can be expanded through strategic chemical selection and testing. Research programs to obtain better quantitative (rate) and qualitative (metabolite) information for chemical biotransformation in a range of organisms are needed. The workshop also highlighted the need for guidance documents on some of the new developments for B criteria.

541 Decamethyltetrasiloxane (L₄): Assessment of biomagnification and toxicity through aquatic/dietary lab studies, fugacity ratio, field and in situ sampling

K.B. Woodburn, Dow Corning / Health and Environmental Sciences; J.A. Kim, Dow Corning Corporation / Health and Environmental Sciences; J.A. Durham, Dow Corning Corp. / Health and Environmental Sciences

Lipophilic chemicals, such as the cyclic and linear methylsiloxanes, are considered possible candidates for potential bioaccumulation (‘B’) in aquatic food webs, and are often found in sediments when present in aquatic ecosystems. As such, risk assessments may be focused on protection of benthic organisms, and probabilistic risk assessment (PRA) methods allow the risk assessor to include stochastic properties of both exposure and response (i.e., toxicity). The objective of this work was to examine laboratory and field data concerning the bioaccumulation and ecotoxicity of decamethyltetrasiloxane (L₄), a linear methylsiloxane. Laboratory bioaccumulation studies have been performed to determine the breakpoint value for L₄ with the guppy (Poecilia reticulata) and the fathead minnow (Pimephales promelas), and the BCF values range from 10 to 3850 L/kg-ww, and the latter study reported an depuration half-life of approximately seven days. Laboratory BCF values for L₄ range from an empirical BMF of 0.04 with goldfish (Carassius auratus) to an kinetic BMF of 3.5 with rainbow trout (Oncorhynchus mykiss). Laboratory bioaccumulation studies have been performed to determine the toxicity of L₄ with the guppy (P. reticulata) and the fathead minnow (P. promelas), and the observed sediment concentrations using a PRA approach. The PRA revealed an absence of overlap of L₄ sediment concentrations (generally non-detactable at

542 Why existing risk assessment tools may get it wrong for hydrophobic organic contaminants: impact of benthic invertebrate biotransformation for P and B

H. Selek, Roskilde University / Dept Environmental Social and Spatial Change

Current risk assessment of hydrophobic organic contaminants is based on these contaminants potential to persist (P) in the environment, bioaccumulate (B) in biota and their potential toxicity (T). This presentation addresses the importance of environmental and physiological factors for affecting the risk of contamination, including biotransformation, of sediment-associated hydrophobic organic contaminants in the aquatic environment and thus the importance of these factors for contaminant persistence. Special emphasis is placed on the 1) impact of benthic invertebrates on fate of sediment-associated contaminants, and 2) variability of biotransformation capacity among benthic invertebrates. In conclusion, even though the bioaccumulation capacity of sediment-associated hydrophobic organic contaminants in the aquatic environment, it is evident that biotransformation may reduce body burden and facilitate the removal of sediment-associated organic contaminants, and especially so in polychaete species thriving in organically enriched and contaminated sediment. Existing risk assessment tools and methods may therefore not be designed to fully address hydrophobic organic contaminants, and evidence point to including benthic invertebrate biotransformation in P and B assessment. By focusing future research on physiological properties that impact accumulation and biotransformation of B and P substances we will be able to reduce uncertainty and thus improve our ability to estimate the first step in B, namely transfer from the abiotic (sediment) to the lower trophic level (i.e., the first step in the benthic-pelagic food web).
Investigating differences in root to shoot transfer and xylem sap solubility of organic compounds between zucchini, squash and soybean using a pressure chamber method

W.J. Donnette, Utah State University / Utah Water Research Laboratory; N. Garvin, Agricultural Engineering

A pressure chamber method was used to examine differences in the root to shoot transfer and xylem sap solubility of caffeine (log Kow = -0.07), triocarban (log Kow = 3.5-4.2) and endosulfan (log Kow = 3.8-4.8) for zucchini (cucurbita pepossp pepo), squash (cucurbita pepo ssp ovifera), and soybean (glycine max L.). Transpiration stream concentration factors (TSCF) for caffeine (TSCF = 0.8) were statistically equivalent for all plant species. However, for triocarban and hydrophobic endosulfan and triocarban, the TSCF values for zucchini (TSCF = 0.6 and 0.4, respectively) were 3 and 10 times greater than the soybean and squash (TSCF = 0.2 and 0.05, respectively). The difference in TSCF values was examined by comparing the measured solubilities of caffeine, endosulfan and triocarban in deionized water to those in soybean and zucchini xylem saps using a modified shake flask method. The measured solubility of organic contaminants in xylem sap has not previously been reported. Caffeine solubilities in the xylem saps of soybean and zucchini were statistically equal to deionized water (2150 mg/L) while endosulfan and triocarban solubilities in the zucchini xylem sap were significantly greater (0.43 and 0.21 mg/L, respectively) than that of the soybean xylem sap (0.31 and 0.11 mg/L, respectively). These results suggest that enhanced solubility of caffeine reported for zucchini is partly due to increased solubility in the xylem sap. Further xylem sap characterization is needed to determine the mechanism of solubility enhancement.

Passive dosing to characterize the sorptive capacities of biota lipids from various trophic levels

A. Jahng, Stockholm University / Department of Cell Toxicology; A.H. Kierkegaard, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; J. Holmåck, R.A. Andersson, Stockholm University / Department of Analytical Chemistry; M. MacLeod, ITM - Stockholm University / Dept of Environmental Science and Analytical Chemistry ACES

In a bioaccumulation context, lipids often are the major partitioning phase for hydrophobic organic chemicals. What is referred to as “lipid”, however, is a complex mixture of triglycerides of neutral lipids (neutral ‘storage’) and polar (“membrane”) lipids. Furthermore, depending on the organism’s fraction of lipids and proteins and the properties of a chemical, another sorptive phases (e.g. proteins) may be particularly important. The aim of the present study was to expand our previous studies of the sorptive capacities of pure lipids into more realistic media, i.e. extractable organic matter (EOM) obtained by solvent-extraction of lipids from various trophic levels. The following steps were part of the experimental design: i) solvent-based extraction of tissue homogenates of five different species: whole blue mussels, herring muscle, pork bacon, guillemot egg and seal blubber; ii) passive dosing of replicates of each EOM sample with the cyclic volatile methyl siloxanes (cVMS) D4, D5, D6, and tri- and tetrachlorobenzene (TrCB and TeCB) via a common headspace for 28-121 hours; iii) sampling of ENVs and olive oil controls at different time points; iv) purge and trap extraction of the model chemicals onto ENV+ SPE cartridges, elution and GC/MS analysis; v) determination of the fractions of storage and membrane lipids in all samples via NMR, SPE followed by LC-ELSD, and GC-FID. The fraction of EOM extracted from the five biota samples ranged from 1.4% (whole blue mussels) to 95% (seal blubber). The equilibrium between the olive oil donor and the EOM samples and olive oil controls in the passive dosing units was fast (in the range of days) for the cVMS. The chlorobenzenes approached, but did not fully reach equilibrium during the experiment. The obtained results showed minor differences of less than a factor of 3 in the sorptive capacities of the EOM obtained from biota of different trophic levels. The olive oil controls generally had higher sorptive capacities for the model chemicals than the EOM samples. The partitioning of the model chemicals between EOM and olive oil was not statistically different for EOM originating from bacon and seal blubber, whereas small but statistically significant differences were found for some chemicals in EOM from blue mussels, herring and guillemot egg compared to olive oil. The highest fractions of polar lipids were found in EOM obtained from blue mussels and guillemot egg, which may partly explain the observed differences.

Exploring the Influence of Molecular Structure on the Membrane Affinity of Organic Bases

s.T. droog, Utrecht University / JRA; N. Timmer, J.L. Hermens, Utrecht University / Institute for Risk Assessment Sciences

Imnobinized Artificial Membrane (IAM) chromatography column was used to determine the phospholipid binding affinity for >100 cationic structures, including primary, secondary, tertiary amines and quaternary ammonium compounds. Eluent conditions were optimized to avoid unwanted electostatic interactions of the silica core of the column particles, resulting in intrinsic Klipw measurements for the cationic species. For several weak bases, IAM measurements performed at pH 3.0, 5.0 and 7.4 allowed for determination of the Klipw for both cationic and neutral species. From these experiments, various correction factors between neutral amine and protonated amine species could be established. New experimental Klipw measurements with solid supported bilayer membranes (TRANSIL) for 30 overlapping cations, and literature comparisons of liposomal partitioning Klipw data for ~5 overlapping cations, indicate that the IAM column is a representative high-throughput method. A first elucidation of how the molecular structure influences the membrane affinity of organic cations is made using ~30 basic amine structures that lack polar functional groups besides a single phenyl ring and the charged nitrogen moiety. The anisotropic structuring of an ordered phospholipid phase appears to be crucial in determining the membrane affinity of cations, regarding the strong differences between linear and branched isomeric structures of the same molecular volume. Using a molecular dynamic simulation of a 3D dissolved membrane bilayer structure, we used COSMOthermXCOSMOmic software calculations that combine quantum-chemical calculations with thermodynamics to visualize the ideal positioning of these basic amine structures in a membrane and compared the predicted Klipw values with IAM experimental data. A second series of amines with various functional groups were compared with the simple amine structures to examine if specific fragment values for each individual functional group can be derived. Additionally, COSMOthermXCOSMOmic calculations for these more complex structures were compared with measured IAM Klipw values.

Developing end-points and effect-based methodologies for characterization of emerging pollutants at relevant exposure concentrations

Ligand Binding Domain of Estrogen Receptor alpha to generate a bioassay for fast detection of Endocrine Disruptor Chemicals

V. Ferrero, L.G.C. Negrao Carvalho, Institute for Environment and Sustainability / Institute for Environment and Sustainability IES; L. Varani, Research Institute in Biomedicine; L. Calzolai, European Commission, Joint Research Centre / Institute for Health and Consumer Protection; I. lettieri, European Commission - Joint Research Centre / Institute for Environment and Sustainability

Endocrine Disruptor chemicals (EDCs) are compounds capable to bind to estrogen receptor (ER). They exert their toxicity to animals and humans by interfering with the normal function of ER, leading to many adverse effects such as reproduction, hormonal and immune system malfunctions, carcinogenesis and feminization in some fish and amphibians. EDCs are commonly released in the environment as results of anthropogenic activities. Their detection and monitoring are still a challenge since the analytical method cannot measure all EDCs at the concentrations usually found in the environment. Analytical measurements are also unable to predict the combined effect of all EDCs present in an environmental mixture. We developed an assay based on recombinant ligand binding domain of human estrogen receptor α for fast detection of EDCs as first screening. If a chemical from the screening shows receptor activity it can be further investigated how the hormone signaling pathways and thus be toxic. Furthermore a rational modification of the ligand binding domain has been designed to generate a mutant receptor to increase the affinity, and therefore the sensitivity towards different classes of compounds (Ferrero VEV et al., 2014). The Ligand Binding Domain (LBD) of the Estrogen Receptor α is highly conserved among human and aquatic species (fish and amphibians) and it is sufficient to bind estradiol and estrogen-like molecules. The encoding gene of the LBD was expressed in E.coli and purified.To test the ability to bind estradiol and other chemicals, an assay was set up using the PolarScreen assay (Life Technologies). We tested the natural ligand 17β-estradiol, its close analog 17α-ethinylestradiol (chosen since it is an emerging pollutant); tamoxifen, an antagonist and three other compounds with known estrogen disrupting activity and significant presence in the environment, bisphenol-A, 4-nonylphenol and 4-tert-octylphenol. An artificial chemical mixture was tested and compared with other ER based assays (Carvalho RN et al., 2014). The Ligand Bindind Domain of the Estrogen Receptor assay (LER) has been conceived for the detection of chemicals which binding to the ER could mean a potential interference with the normal activity of the receptor in the organisms. The assay is fast and easily handled and it can be used as first screening to measure the EDCs in matrices e.g., water, soil and food.

Assessing the cytotoxic, estrogenic and mutagenic effects of pharmaceutical residues and their photo- transformation products in water

M. I. Vasquez, University of Cyprus / Department of Civil and Environmental Engineering University of Cyprus; M. Tarapoulou, NIREASIWRC / Department of Civil and Environmental Engineering University of Cyprus; N. Lambrianides, The Cyprus Institute of Neurology and Genetics; K. Felekiss, University of Nicosia / Department of Life and Health Sciences; C. Sticht, M. Saile, N. Gretz, Heidelberg University / Medical Research Center Faculty for Clinical Medicine Mannheim; D. Fatta-Kassinos, University of Cyprus / Nereus International Water Research Center
Pharmaceuticals are now considered as environmental contaminants. Their presence has been reported in the aquatic and terrestrial environment, as parent compounds, metabolites and/or transformation products. The adverse effects to biological systems caused by the exposure to these compounds are yet to be thoroughly investigated and fully understood. To this end, the purpose of the present study was the assessment of the cytotoxicity, estrogenicity and mutagenicity of eleven pharmaceuticals namely azithromycin, erythromycin, ofloxacin, sulfamethoxazole, diazepam, ibuprofen, atenolol, metopropl and propranolol. A selection of photolabile active pharmaceutical ingredients was performed to further assess the effects of transformation products that may occur in the environment. Diclofenac, ofloxacin and sulfamethoxazole were photolytically treated in order to investigate the cytotoxicity, estrogenicity and mutagenicity of the mixture of their transformation products on mammalian and human cells. A screening of the gene expression profile was carried out as a supplementary endpoint in order to reveal the mode of action of these transformation products to the human genome. The main findings indicate that mutagenicity was not observed at concentrations lower than 50 μg/L. Interestingly, synergistic effects were observed at an exposure concentration of 10 μg/L to the mixture of pharmaceuticals. No significant estrogenic effects were observed to any of the pharmaceuticals and the mixtures assessed. The gene expression profile was affected to a greater extent by the mixture of diclofenac, ofloxacin and sulfamethoxazole. 644 genes were differentially expressed in the cells treated with the pharmaceutical mixture and 216 were expressed in the cells treated with the irradiated pharmaceutical mixture. 466 genes were expressed in both types of exposure. The most important pathways influenced were the MAPK signalling, the cell cycle, the p53 signalling and the protein processing in endoplasmic reticulum.

The present proposal offers an alternative testing system that sheds light to less studied aspects related to the exposure to pharmaceuticals. Mutagenicity, estrogenicity, cytotoxicity and gene expression profiling could potentially be used in future studies for the assessment of effects in real matrices.

548 Identifying drivers of chemical mixture’s bioactivity at realistic environmental concentrations?
L Rodríguez-Palomares, Universidad Autónoma de Madrid / Biology; R. Muñoz-Carpena, University of Florida / Department of Hydrology Water Quality Agricultural Biological Engineering Faculty; S. Gonzalo, Universidad de Alcalá; M. González-Pileier, Universidad Autónoma de Madrid; R. Rosal, Ingeniería Química; F. Fernandez-Pitius, Universidad Autónoma de Madrid / Biology Current methodologies applied to characterize the effect of suspect substances, such as environmental metabolites (EMs), do not provide relevant information on the concentration effect on the mixture. A systematic screening approach was developed.

In the present work we applied a new experimental framework for the effect-based identification of hazardous EMs. The methodology we denominated as GSUA-EDS, consists in incorporating Global Sensitivity and Uncertainty Analysis (GSUA) tools to assist Effect-Directed Screening (EDS) of suspect substances in ecotoxicological studies. GSUA tools are top-down approaches which serve to identify important factors driving a system’s response independently of any mechanistic assumption, such as linearity, additivity or mode of action (MoA). In the present study, we used the global sensitivity computational screening tool known as Morris Method [2] to study main drivers controlling low-dose responses of an in-vitro ecotoxicological test when exposed to realistic mixtures of 16 pharmaceuticals. The effect of one environmental stressor (light intensity) was also included in the analysis as an example of the open possibilities that GSUA-EDS offers to environmental ecotoxicologists. A total of 1984 doses were tested with 3 dose levels of 14 pharmaceuticals. The results obtained were compared to the literature. From the data, a mixture of both types of exposure was selected for further studies in zebrafish. Based on the assessment of different chemical endpoints following different exposure periods, our study also aims to analyze proteome alterations in exposed zebrafish embryos using mass spectrometry-based Multidimensional Protein Identification Technology (MudPIT) in order to investigate molecular mechanisms of tralopyril toxicity and find novel biomarkers of exposure.

Furthermore, a LC-MS/MS method was developed allowing measurement of tralopyril concentrations in the exposure medium.
proper parameterization for “data poor” compounds. In order to expand the
capability of PBPK models to cover as much as possible the chemical space,
model parameterization for data poor chemicals is done using advanced
quantitative structure-activity relationships (QSARs). Several QSAR modeling
approaches have been investigated, including (a) an algorithm based on fractional
content of and the lipophilicity of the compound of interest; (b) the molecular
fractions algorithm that takes into account the frequency of occurrence of the
several molecular fragments of the compounds and (c) Abraham’s solvation
equation for estimating biological properties, which takes into account the excess
molar refraction, the compound dipolarity/polarizability, the solute effective or
summation hydrogen-bond acidity, the solute effective or summation
hydrogen-bond basicity and the McGowan characteristic volume. Up to now, these
QSAR approaches have been applied to a limited number of chemical families. A
major breakthrough came from the use of Artificial Neural Networks coupled
to Abraham’s solvation equation for predicting chemical-specific
biological/biochemical properties such as blood-tissue partition coefficients,
maximal velocity (V_max) and the Michaelis - Menten constant. This was
a remarkable advance, since the prediction capability of the Michaelis - Menten
constant in the existing studies to date was rather poor (R² 0.3). With our coupled
ANN - Abraham’s solvation equation method for the investigated group of 55
chemicals, R² rose to 0.8. For the rest of the parameters (partition coefficients,
V_max), performance of prediction against experimental values was consistently high
(R² always above 0.9), outperforming any other existing methodology. Overall, the
proposed methodology, offers two major advantages: - It helps filling the data gaps
of data poor chemicals, acts as an input agent and is well understood as: a wide
array of chemical classes. - It effectively supports the “safe by design” concept for
industrial chemicals, by allowing the successful prediction of toxicokinetic
behavior based on molecular parameters, thus avoiding chemical structures
resulting in bioaccumulative and non-rapidly metabolized chemicals.

552 Computer Aided Discovery and REDesign of Skin Sensitizers - a rational
modeling approach of the skin sensitization AOP that provides guidance to
molecular redesign of sensitizing chemicals

J. Kostal, Toxifx LLC; A. Vountchova-Kostal, George Washington University / Chemistry Department

Designing molecules from first principles to have predictable biological activity is
still viewed as an immense challenge; however, recent advances in mechanistic
toxicology and computational chemistry can provide great insight into minimizing adverse biological effects. Computational chemistry has revolutionized drug discovery over the past two decades by providing rational means to optimize biological activity of small molecule drugs through changes to the molecular structure. Some of the same methods can be used to minimize activity of commercial chemicals against specific biological targets. The CADRE approach capitalizes on the insight provided by drug discovery and modeling of organic and enzymatic reactions. The CADRE-SS variant assesses the skin sensitization potential of chemicals, and informs design of safer alternatives to replace potent sensitizers. Skin sensitization is a major concern for the chemical industry. NMs can be found in a large variety of applications and some
have the scope for an almost limitless range of options in this. Thereby, the
available experimental data were filtered and used to build
silico models can be applied in the hazard assessment procedure to
evaluating the potential environmental hazard of NMs. The present study fits this
framework, aiming to assess the influence that functionalization can have in the
ecotoxicity of seven variations of soft organic NMs made of biosurfactants. These
later found to poorly comply with environmental regulation. The present study fits
the necessary arena has been built to avoid the errors made in the past by the classic
chemical industry. NMs can be found in a large variety of applications and some
have the scope for an almost limitless range of options in this. Thereby, the
available experimental data were filtered and used to build
silico models can be applied in the hazard assessment procedure to

the nanosurfactants to L. minor: an increase in the number of EO units seems to reduce NMs toxicity. Further studies are needed to fully understand how NMs functionalization may help nanotechnology to create more environmentally friendly solutions while retaining their desired functions.

556 Estimation of Octanol Water Partition Coefficients and Skin Permeability Exclusively from Spectroscopic Data
A. Voutchkova-Kostal, George Washington University / Chemistry Department; N. An, George Washington University / Department of Chemistry

The rational design for safer chemicals requires tools for predicting bioavailability and toxicity that can be used seamlessly by chemists alongside traditional synthesis and characterization. We have developed a set of Quantitative Spectroscopic Data-Activity Relationship (QSDAR) models that predict octanol/water partition coefficient (logP) and skin permeability (logKp) of organic chemicals using only NMR spectra. The advantage of these models over traditional structure-based in silico methods is that (a) they do not require knowledge of exact structure and (b) they are applicable to mixtures and formulations in addition to pure chemicals. The models also have excellent predictive power (R² > 0.85 and Q² > 0.75) and have been thoroughly externally validated. Each model has been trained on a large training set with highly diverse structures.

557 EFSA 'Risk Assessment for Birds and Mammals' guidance document - the future
R. Sharp, EFSA - European Food Safety Authority / Pesticides Unit; M. Arena, EFSA - European Food Safety Authority / Pesticides; D. Auteri, Auteri / Pesticides Unit; A. Ippolito, EFSA - European Food Safety Authority / Institute for Environment and Sustainability IES; F. Streissl, EFSA / Pesticides Unit; C. Szentes, Pesticides Unit

In order to gain EU Level approval of an active substance under Regulation (EC) 1107/2009, it is necessary to demonstrate that there are no unacceptable risks to bird and wild mammal species. Since 2008, EFSA has been developing a series of guidance documents to aid the risk assessor. These guidance documents are intended to assist risk assessor in their work in a structured and methodological way. However, practice in its use has identified certain aspects of the guidance documents which often require careful interpretation and often lead questionable proposals being made in the risk assessments presented in the dossiers of the Draft and Renewal Assessment Reports (DARs/RRAs). This presentation will provide an analysis of common problems which are encountered when performing a risk assessment using the EFSA (2009) guidance document, discuss areas of the guidance which have been identified to be in need of improvement and also reflect on areas which are working well. The plan for the update of the guidance document will be discussed and, if possible, the timelines will be given.

558 Industry view on the implementation of the EFSA Guidance Document on Birds and Mammals in the EU
J. Pascual, BASF SE; R. Murfitt, Syngenta Ltd / Environmental Safety; K. Brugger, DuPont Crop Protection; T. Carro, DuPont Crop Protection / Environmental Safety Assessment; M. Ebeling, Bayer CropScience AG EnSa-ETX/TV - Environmental Safety and Ecotoxicology; J.P. Edwards, Central Product Safety Dept; M. Fouodoulakis, Dow Agrosciences / RSRA ERS; T.B. Fredricks, Monsanto Company / Zoology; B. Garlej; J. Jahnke, Bayer CropScience AG; P. Marsan, Cheminova ÅS / European Regulatory Office / Global Regulatory Science

EFSA’s Guidance Document on the Risk Assessment for Birds and Mammals (EFSA Journal 2009 7(12):1438) (EFSA GD) was the first guidance document in the area of ecotoxicology developed by EFSA. Several aspects, both scientific and regulatory, of the new GD were highly debated at the time of discussion and approval of the document by European officials before its official implementation in July 2010. After almost 5 years of working on parameters used for refined (higher tier) risk assessments according to the EFSA GD. Among these are: selection of focal species representative of different crops and regions; risk assessment for voles and associated regulatory decisions by regulators; selection of reproductive endpoints from laboratory studies for refined risk assessments for wild birds and mammals; residues in food items; data availability and analysis for Proportion of Time (PT) data; role of field effects studies as top tier studies for regulatory decisions and current role and acceptability by regulators of modelling.

559 Use of post-authorization field monitoring data to assess the availability of cereal seeds on the soil surface after drilling
E. Bonneris, R. Barkuech, Bayer CropScience Aktiengesellschaft, BCS AG-D-EnSa-ETX; P. Adrian, CEHTRA SARL; R. Grau, Bayer CropScience / D-GRS

The risk assessment for Plant Protection Products (PPP) on Birds and Mammals is currently performed under the EU Regulation (EC) 1107/2009 and its amendments. This guidance document issued by EFSA (EFSA 2009[1]). The first tier bird and mammal risk assessment for seed treatments assumes that the diet of granivorous species consists entirely of readily available, freshly treated seeds. Many pesticides, used as seed treatments, fail to meet the standard regulatory triggers for acute and long-term risk to birds and mammals and hence require refinement. At higher tier, after selection of suitable focal species, the risk is contextualised by estimating the area that would need to be foraged for a bird or mammal to exceed the acceptable dose. Indeed, different options for refinement are available, including field studies and monitoring surveys where exposure to remaining treated seeds is assessed. Currently, there is limited published information on the density of seeds available on the soil surface, which covers the various agricultural practices of farmers during drilling in Europe. Post-authorization field data obtained in Europe (i.e. > 0.75 ha) in different locations covering the main regions cultivated for cereal production in Europe. The program was also designed in order to assess the possible relationship between seed availability and different agronomic parameters. Variables such as soil humidity, presence of stones, soil type, preparation of the soil prior seeding, drilling depth, drilling speed, and seed density were recorded and evaluated. The outcome of this large-scale monitoring exercise will be compared to and interpreted in perspective of existing literature on the subject and in taking into consideration the current exposure assumptions used in the bird and mammal risk assessment. [1] European Food Safety Agency; Guidance Document on Risk Assessment for Birds and Mammals and honey bees. EFSA Journal 2009 7(12):1438 [358 pp.]; Available online: www.efsa.europa.eu

560 Arena studies offer a more realistic worst case scenario test system than conventional housing for Wood mouse ecotoxicology studies
N. Dennis, S. Milner, K. Tarrant, Food and Environment Research Agency / Centre for Chemical Safety and Stewardship

Behavioural and consumption studies using singly housed small rodents, such as Wood mice (Apodemus sylvaticus), are routinely conducted in small cages such as rat/hamster cages that are approximately 20cm wide, 45cm long and 21cm high. Larger and larger in size (i.e. > 0.75 ha) may be large enough to provide a more realistic test system. This test system enables Wood mice to express more natural foraging behaviour over a larger area that is more representative of the natural environment. Wood mouse activity levels are much higher than in smaller cages and therefore energy demands are more characteristic of wild populations. Recent research conducted by Fera have found that Wood mice respond to the challenge of feeding in an arena, which is a more realistic test system than conventional housing. The arena studies offer a more realistic worst case scenario test system than conventional housing for Wood mouse ecotoxicology studies.
ranges overlapped partially between consecutive sessions (mean 40%, range: 9–73%). In both crops, size of daily home range was stable regardless of CP-application. After application, in both crops, GT spent less time foraging inside the treated area. Also the proportion of treated area within the home range decreased. Travelled distance was unaffected. After application, BB spent more time foraging in citrus. Contrarily, after application, in UK apple orchards BB reduced the amount of time foraging and the travelled distance increased. These differences for BB between grape and UK-apples might be due to attractiveness of surroundings. Surroundings of citrus were drier and less attractive for feeding than moister conditions within the citrus which is drip-irrigated. This was different to the wetter off-crop conditions in UK orchards. Only in citrus were trees suitable for BB to nest. If nesting, birds might prefer to keep foraging areas closely. For both species the amount of time was drier before and during CP-application but not after. Overall, birds did change their foraging strategy. Food depletion caused by the insecticide was compensated by spatial change of the foraging area rather than change in diet. Birds used parts of their daily home ranges constantly and other temporarily. This flexibility allowed adaptation to a temporary reduction in arthropod biomass following insecticide application.

562 Key drivers of avian breeding-success in chlorpyrifos-treated citrus (Spain) and apple orchards (UK).
R. Dittrich, Tier3 Solutions GmbH / Wildlife Ecology; Z. Gao, F. Sotti, B. Giessing, Tier3 Solutions GmbH; S. Norman, RidgewayEco; N.N. Poletika, Dow AgroSciences LLC / Field Exposure and Effects Department; C. Wolf, Tier3 Solutions GmbH

It is challenging to balance environmental effects of pesticides with the need for food. Hence, it is important to assess the reality in the field and measure directly the effects from actual use to reach evidence-based decisions. Our study evaluated factors which influence reproduction of birds nesting in citrus (Spain) and cider-apple orchards (UK) including spraying of broad-spectrum insecticide chlorpyrifos (CP) in the breeding season. Potential influence of CP in comparison with effect of habitat, ecological factors, and weather was assessed. Reproduction was measured by counting active nests and no. of fledglings. Effect of predation was explored. Nest searches were conducted in 3 to 4 consecutive years. No. of sites varied from 5 to 10 depending on country & year. Nest boxes (500 per country) were installed to give insectivores Great Tit and Blue Tit increased chance to breed in orchards to maximise potential to detect effects of CP. Where nests were empty earlier than expected all signs (shells, body parts, tracks of predators) were recorded to judge cause of failure. Cameras were used for some nests. A logistic−exposure model was applied to identify key drivers of nest success. Citrus/Spain: 602 nests of 14 species were monitored. Most common were Blackbird, Serin, Greenfinch and Great Tits. A third of nests were successful. Great Tits had highest breeding success with mean of 4.3 and 3.4 young per nesting attempt in 2011 and 2012. For open nesters, e.g. blackbird, 0.7 and 1.2 young per nesting attempt were found. Some nests were destroyed by pruning. In citrus, CP-application is relatively late in breeding season. Hence, only 18% of nests was exposed. For Blackbird, nest survival probability was decreased in two sites by CP-application, but this species still reproduced successfully and was abundant. Apples/UK: in 2012, nests of 19 species were found in orchards (n = 70) and surroundings (n = 53). 64% inside orchards successfully fledged while 47% in surroundings were successful. With nests boxes, breeding attempts in orchards increased to 153 in 2013. In 79% of nests inside orchards, fledglings were produced. In surroundings 31% of attempts were successful. Inside orchard 4.9 young per nesting attempt and in nests found in surroundings 2.2, young were produced. Overall, predation of nests and increasing date of breeding-attempt were found to be the key drivers of breeding-success in citrus and cider-apple orchards.

Risk assessment, risk management and mitigation for pesticides: from regulation to public perception (III)
A. Alix, Dow AgroSciences / Risk Management; C. Garrido, BeeSafe; B. golln, Institute for Strategies and Technology Assessment; M. Miles, Bayer CropScience UK / Environmental Safety

The registration process for Plant Protection Products (pesticides) in agriculture relies on a preliminary evaluation of the risks they may pose to human health and the environment, among which honey bees and other non-target arthropods in the farmland [1]. If necessary, specific risk mitigation measures may accompany the registration in providing detailed conditions of use to reduce pollinators’ exposure [1]. In addition, the reform of the European Common Agriculture Policy (CAP) includes the implementation of ecological focus areas, which should represent at least 5% of the arable area of the holding for farms with an area larger than 15 hectares (excluding permanent grasslands) [1]. In both sectors, the need for mitigation measures is necessary, aim at achieving this goal. These risk mitigation measures are reported on the labelling of pesticide products [3]. The ecological focus areas presented in the CAP aim at restoring or preserving biodiversity in the farmland. Therefore although the measures adopted may take different forms, they are targeting a common benefit on biodiversity, which has therefore been measured in a number of studies to evaluate the efficacy of farmland management plans at improving biodiversity in cultivated areas [4]. This presentation will illustrate the outcome of the review dedicated to the efficiency of risk mitigation measures at preserving and/or restoring biodiversity in cultivated areas, undertaken during the MagPie workshop, and discuss this outcome in relation to risk mitigation measures and risk assessment applied to pesticides.

565 Pesticide risk mitigation at landscape level (RISKMIN) - A spatial approach to maintain and develop agrobiodediversity
B. Scholz-Starke, RWTH Aachen University, Institute for Environmental Research and Analysis of Agricultural Ecosystems, Germany; B. Scholz-Starke, RWTH Aachen University, Institute for Agroecology; M. Deubert, L. Streib, RLP AgroScience GmbH; A. Toschki, Research Institute gaiac; C. Kula, Fed. Office for Consumer Protection & Food Safety; M. Roß-Nickoll, RWTH Aachen University / Institute for Environmental Research

 Nowadays, it is widely accepted, however controversially discussed, that species diversity and the quality of habitats generally decrease with increasing intensity of agricultural land-use. Therefore the current off-crop mitigation tools to avoid unacceptable risk from the use of pesticides are critically reviewed. Complementary landscape level mitigation options to protect non-target biodiversity in Germany are considered. An approach is presented that considers the real landscape structure by classifying the landscape elements according to their biodiversity of various arthropod groups. The project RISKMIN includes an ‘ecological value’ depending on spatial distribution of typical elements of terrestrial agricultural landscapes in Europe, exemplified by two regions in Germany. The assessment of the efficacy of landscape-level mitigation measures, such as extensification or providing a higher variety of structural elements is analysed. The RISKMIN project is organised as a modular and interdisciplinary approach. Firstly, the module GEODAT defines 50 types of ecologically relevant landscape elements (LE-types). To each of the elements an ecological value between 0 and 100, derived from impact regulation closely related to the biotope type is assigned, which reflects the natural value of the respective element. Advanced remote sensing using geographic information systems identifies the shapes of landscape elements and collects (geo-) data that is necessary to allocate LE-types to the shapes. An integrated landscape ecological value is calculated as the sum of all ecological values of the single LE module (STATUS QUO), which is then corrected for intensive agricultural use for LE nearby in-crop areas. The module PROJECTION then identifies and defines most promising risk mitigation measures (RMM) of different mechanisms and implements rules for mechanisms of mitigation at landscape level. The methodology is considered well established but needs calibration by data from different landscapes. The model that was derived from the RISKMIN project puts the discussion on landscape-based RMM a step forward. We implement the fact that intensive agricultural practices come along with undesired side-effects in the long term and thus lead to a significant downgrade of the landscape status compared

564 Biodiversity in monitoring studies investigating the efficacy of risk mitigation measures in the farmland
A. Alix, Dow AgroSciences / Risk Management; C. Garrido, BeeSafe; B. golln, Institute for Strategies and Technology Assessment; M. Miles, Bayer CropScience UK / Environmental Safety

The registration process for Plant Protection Products (pesticides) in agriculture relies on a preliminary evaluation of the risks they may pose to human health and the environment, among which honey bees and other non-target arthropods in the farmland [1]. If necessary, specific risk mitigation measures may accompany the registration in providing detailed conditions of use to reduce pollinators’ exposure [1]. In addition, the reform of the European Common Agriculture Policy (CAP) includes the implementation of ecological focus areas, which should represent at least 5% of the arable area of the holding for farms with an area larger than 15 hectares (excluding permanent grasslands) [1]. In both sectors, the need for mitigation measures is necessary, aim at achieving this goal. These risk mitigation measures are reported on the labelling of pesticide products [3]. The ecological focus areas presented in the CAP aim at restoring or preserving biodiversity in the farmland. Therefore although the measures adopted may take different forms, they are targeting a common benefit on biodiversity, which has therefore been measured in a number of studies to evaluate the efficacy of farmland management plans at improving biodiversity in cultivated areas [4]. This presentation will illustrate the outcome of the review dedicated to the efficiency of risk mitigation measures at preserving and/or restoring biodiversity in cultivated areas, undertaken during the MagPie workshop, and discuss this outcome in relation to risk mitigation measures and risk assessment applied to pesticides.

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564 Biodiversity in monitoring studies investigating the efficacy of risk mitigation measures in the farmland
A. Alix, Dow AgroSciences / Risk Management; C. Garrido, BeeSafe; B. golln, Institute for Strategies and Technology Assessment; M. Miles, Bayer CropScience UK / Environmental Safety

The registration process for Plant Protection Products (pesticides) in agriculture relies on a preliminary evaluation of the risks they may pose to human health and the environment, among which honey bees and other non-target arthropods in the farmland [1]. If necessary, specific risk mitigation measures may accompany the registration in providing detailed conditions of use to reduce pollinators’ exposure [1]. In addition, the reform of the European Common Agriculture Policy (CAP) includes the implementation of ecological focus areas, which should represent at least 5% of the arable area of the holding for farms with an area larger than 15 hectares (excluding permanent grasslands) [1]. In both sectors, the need for mitigation measures is necessary, aim at achieving this goal. These risk mitigation measures are reported on the labelling of pesticide products [3]. The ecological focus areas presented in the CAP aim at restoring or preserving biodiversity in the farmland. Therefore although the measures adopted may take different forms, they are targeting a common benefit on biodiversity, which has therefore been measured in a number of studies to evaluate the efficacy of farmland management plans at improving biodiversity in cultivated areas [4]. This presentation will illustrate the outcome of the review dedicated to the efficiency of risk mitigation measures at preserving and/or restoring biodiversity in cultivated areas, undertaken during the MagPie workshop, and discuss this outcome in relation to risk mitigation measures and risk assessment applied to pesticides.
to past reference state. The RISKMIN concept recommends options for risk managers especially to optimize RMM alongside with national programs for sustainable land use.

566 Can we make balanced decisions? Always look on the bright cider life? S. Deacon, ENVIRON UK Ltd; S. Norman, RidgewayEco; M. Rockel, ENVIRON International Corp.; P. Burston, ENVIRON; L. Sanderson, N. Eary, ENVIRON UK Ltd; F. Crundle, Dow AgroSciences LLC

Regulators at EU and Member State level face the challenge of having to make decisions on plant protection products (PPP) using environmental risk assessments, which do not include information on benefits. In particular, evidence on how a particular plant protection product affects the socio-economics of farming is currently not part of the decision-making process. Regulation 1107/2009 states ‘The purpose of this Regulation is to ensure a high level of protection of both human and animal health and the environment and at the same time to safeguard the competitiveness of Community agriculture’. The latter element is recognition that a regulatory decisions on a PPP should not made ‘in a vacuum’, especially if there is no evidence of harm to non-target populations in the field resulting from its use according the Good Agricultural Practice. The authors have undertaken a number of cost-benefit analyses to evaluate the use of a range of PPPs and crops. Their approach provides a holistic basis for making pragmatic and practical decisions in a transparent manner. When maximising agricultural land for food production, ecosystem services analysis and socio-economic assessment of farming can enhance farming. To evaluate the toxicity of these plant extracts to environmental, social and economic evidence. The paper gives an overview of potential implications of not taking holistic decisions. For example, growers rely on crop protection tools. When this ‘toolbox’ is threatened by regulatory decisions, pest resistance, and new policies based on hazard instead of risk, then products may be removed from the market. If there is no alternative active substance or if the outcome is an over-reliance on one mode-of-action – leading to pest resistance – cultivation of some crops may no longer be competitive. This can lead to changes in practices or even land-use itself. These changes also influence ecosystem services, which may be decreased – i.e. a worse situation - compared with before the regulatory action. The use of ecosystem services in PPP regulation will be presented and illustrated by a case study where the use of an insecticide in cider apple production in UK has been evaluated.

567 Risk Assessment on Natural Organisms of Organic Farming Materials from Plant Extracts for pest Control in Korea

L. Lee, NAAS, RDA / Erogsafety; K. Park, NAAS, RDA / Chemical Safety Division; M. Paik, NAAS RDA; S. Han, RDA; J. Jin, NAAS RDA; B. Kim, National Academy of Agricultural Science; S. Hong, National Academy of Agricultural Science / department of crop life safety; N. Cho, NAAS RDA

This study was performed to evaluate the risk of the organic farming materials for insect control. In South Korea, neem, sophora and derris extracts are used mostly for organic farming. To evaluate the toxicity of these plant extracts to soil life, Eisenia fetida, was conducted some tests, fish acute toxicity test, honeybee acute toxicity tests, honeybee toxicity test of residue on foliage and earthworm acute toxicity test. According to the acute toxicity tests, neem and derris extracts were very low in toxicity to Cyprinus carpio, Misugniesz anguiuicudatius, Apis mellifera L. and Eisenia fetida, Abycon some tests, fish acute toxicity test, honeybee acute toxicity tests, honeybee toxicity test of residue on foliage and earthworm acute toxicity test. These changes also influence ecosystem services, which may be decreased – i.e. a worse situation - compared with before the regulatory action. The use of ecosystem services in PPP regulation will be presented and illustrated by a case study where the use of an insecticide in cider apple production in UK has been evaluated.

568 State of the art in mechanistic effect modelling for aquatic risk assessment of plant protection products

T.G. Preuss, Bayer CropScience / Environmental Modelling; h. beveclo, Environmental Risk Assessment Team; A. Focks, Alterra Wageningen University and Research Centre /Eco-toxicology; Environmental Risk Assessment Team; U. Hommen, Fraunhofer IAME; T. Strauss, Research Institute gauc / Research Institute Gaia; P. Thorbek, Syngenta / Environmental Safety

Mechanistic effect modelling (MEM) is becoming increasingly important for environmental risk assessment and environmental science. The advantages of MEMs are manifold: They enable the extrapolation of effects between different exposure scenarios, they are necessary for the development of new test methods or test results (e.g. toxic effects to fish and algae), they enable a better understanding of the effects of pesticides and they allow the ecotoxicologist to gain a deeper understanding of the exposure situations. In this way, the exposure situation can be described in a more realistic way and the ecotoxicologist is able to make more accurate predictions. The aim of the current presentation is to give an overview on the achieved milestones within the mechanistic effect modelling for aquatic risk assessment. Therefore the available models for aquatic risk assessment will be reviewed based on their validation status, their similarities and differences and their possible use in the risk assessment. Appropriate modelling approaches have to be selected depending on the risk assessment objective (e.g. time to recovery of populations, effects on individual level) as well as on the level of biological organisation, namely the individual, population or community level. Therefore the model approaches have to be chosen in line with the specific protection goals as defined by EFSA. In order to choose the right modelling approach it is also necessary to consider the data requirements which is the basic dataset we have to address these specific protection goals. We here present models for all organism groups defined in the aquatic guidance document and show the potential use of these models for common risk assessment questions, like extrapolation from lab to field, from individual to population, to untested exposure situation and to untested ecological scenarios. Here the state of the arts model approaches will presented and linked to their domain of applicability in environmental risk assessment of plant protection products. We will demonstrate what can be learned from the models developed so far and suggest a more generic modelling framework. Mechanistic effects modelling will help to incorporate the complexity of the real world into our environmental risk assessment and thereby will be an necessary tool to foster the debate on needed complexity and to set protection goals.

Commute air quality in rail subway systems: current understanding and future mitigation

569 Public transport and infection spread: is this a problem?

L. Morawski, Queensland University

570 Bioaerosol exposure in subway trains and stations

C. Duchaine, Universite Laval

571 Subway particles: what do we breathe on platforms and in trains?

M. Pires, Geosciences; V.I. Martins, M. Mingullion, C. Roche, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); E. de Miguel, M. Capdevila, S. Centelles, Transports Metropolitans de Barcelona (TMB); X. Querol, Institute of Environmental Assessment and Water Research (IDAEA-CSIC)

Commuting by underground rail is a transport mode used daily by over 100 million people worldwide. Published reviews of subway air quality worldwide reveal a wide range of PM concentrations present in underground platforms, and pose the question: why is there such diversity? The air quality of a given subway platform involves a complex interplay of the ventilation system, station depth and design, train speed, frequency, wheel materials and braking mechanisms, and number of passengers being transported. Air movements on the platform show constantly repeated cycles involving turbulent flow through some combination of mechanically forced ventilation systems, blast shafts, and platform access points, driven to a large extent by the piston effect of the trains moving through the tunnels. Narrow platforms with single-track tunnels are strongly dependent on forced tunnel ventilation, in contrast in wider stations with spacious double-track tunnels ambient PM levels can actually improve when tunnel ventilation is switched off, offering the possibility of significant energy savings without damaging air quality. Another variable is that occurring spatially along the platform, with the greatest accumulation of PM usually occurring at one end, depending on station design. Access tunnels can help dilute PM concentrations by introducing cleaner air from outside, although lateral accesses are less effective than those at the train entry point. More interesting than the mass concentration of PM is the fact that platform particles are pervasively ferruginous and commonly accompanied by other elements such as C, Si, Ca, Mn, Cr, and Ba. They are both physically and chemically different from the typical outdoor urban cocktail of inhalable PM breathed above ground in the city. These subway particles display a spectrum of morphologies but by far the most common occurs in the form of irregular, rough-surfaced flakes measuring at most just a few microns in size, commonly displaying cracked and corroded textures. Thus, the dominant mix of source materials and processes of PM generation are unique to the subway environment. This work is supported by the Spanish Ministry of Economy and Competitiveness and FEDER funds (CGL2012-33066, METRO), EU (FP7 grant 315760 HEXACOMM) and IMPROVE LIFE project (LIFE13 ENV/ES/000263).

572 Measuring PM2.5, particle number and black carbon concentration on the London Underground

F. Kelly, Kings College London; J. Smith, D. Green, S. Bevers, B. Barratt, Kings College London / MRCPH, Centre for Environment and Health

The London Underground is a mostly subterranean railway system consisting of 402 km of track that carries over 1.2 billion passengers per year in the Greater London region (Transport for London, 2014). Passenger exposure is under-researched, limited to occupational-health research which has focused on drivers’ shift-time exposure, and a small number of studies typically focused on comparing urban transport modes. Adams et al. (2001) sampled on the Bakerloo, Northern and District lines measuring PM2.5 concentrations. Seaton et al. (2005) sampled on the Central, Northern and Victoria lines, and at three stations, measuring PM2.5 and particle number concentration. In a new study we placed personal monitoring equipment (Aethlabs AE51 microAeth, TSI Sidepak AM510 and Phillips Aerosense Nanotracer) in the driver’s cabin, and simultaneously in the...

573 Air Quality in Subway Stations with Dynamic Control of Energy A. Giretti, Polytechnic University Marche

574 LCA of nanotechnology-based products

574 Nanomaterial release data for LCI and derivation of nanomaterials impact factors for LCA: Nanopolytox case studies D. González, Leitat Technological Center; C. Hidalgo, Leitat Technological Center / BD Safety; Sustainability Division; G. Janer, Acondicionamiento Tarrasaense; E. Fernández-Rosas, G. Vilar, Leitat Technological Center; M. Escamilla, LEITAT/Sustainability Unit; S. Vázquez-Campos, Safety Sustainability

The increasing use of nano-enabled products has brought controversy due to the lack of data on their potential impact on human health and environment. On that concern, there is a consensus that Life Cycle Assessment (LCA) is a suitable method to assess the overall performance of this new technology, but the number of studies that generate data for the Life-Cycle Inventories (LCI) is very scarce, most of them focus only in the manufacturing step (cradle-to-gate studies) and the impacts of released NM are usually neglected. So, LCA for nanotechnologies is challenging since there still are a lot of uncertainties and data gaps. The NanoPolyTox project was designed to propose a methodology to determine the Human Health (HH) and Freshwater Ecosystem (FE) impacts due to the release of NM during the whole nano-enabled product value chain; further it will develop this methodology to derive the impact factors of nanocomposite applications; and, finally, include these impact factors in a complete cradle-to-grave LCA study. In order to determine such HH and FE impacts, a combination of release determination over the different life-cycle stages (NM synthesis, nanocomposite production, use and recycling/disposal) and characterization factors was performed for three polymeric nanocomposites for outdoors applications (MWCNT-PP, TiO₂-PA, ZnO-EVA). NM releases were determined based in the literature for the manufacturing and experimentally for the other stages. Accelerated aging conditions were used to evaluate the release of nanomaterials and possible transformations during the use phase, and recycling and end-of-life processes were also evaluated to evaluate the transformations occurring on the nanomaterials. For the characterization factors, USEtox® was used as starting point; we have developed a general methodology to derive factor, human health and ecotoxicological factors for nanomaterials. On one side, fate and intake models were adapted to NM exposure factors determination. On the other, different approaches were considered for human health hazard and ecotoxicological factors derivation depending on the availability of data. Different variables can dramatically change the hazardous effects of a nanomaterial. Therefore, decision criteria were suggested on how to select input data for these factors. Despite these particularities, the general concept and methodology around the derivation of human and ecotoxicological effect factors are however unchanged.

575 Comprehensive LCI and LCIA modelling for nanomaterials and its influence on study results – the case of Nano-TiO₂ R. Hirschier, EMPA / Technology and Society Lab; B. Salieri, EMPA / C.I.R.S.A; M. Pini, University of Modena and Reggio Emilia / Department of Sciences and Methods for Engineering

A couple of review studies in the last 5 years (e.g. Hirschier and Walser, 2012; Miselec and Olsen, 2014) have shown, that the application of LCA in the area of nanotechnology currently presents three major weaknesses and thus challenges: (i) the choice of an adequate functional unit, taking into account all functionalities that a new product version, containing engineered nanomaterials (ENM), shows in comparison to a traditional option, (ii) the availability of adequate, consistent and complete life cycle inventory (LCI) data for the production of the applied ENM, and (iii) the availability of impact assessment characterisation factors, especially concerning human toxicity and ecotoxicity for releases of nanoparticles along the life cycle. However, so far the question is open, which of these three above mentioned challenges / points has the biggest influence on the results of an LCA study; LCA study in which not the ENM, but the application case is the central view of investigation. This question is investigated by using the case of nano-TiO₂; one of the most relevant and most sold ENM on the market (Piccinno et al., 2012) – and in the same time one of the most comprehensively examined ENM with LCA (see e.g. Pini et al., 2013, Salieri et al., 2015, Pini et al., 2015, or Hirschier et al., 2015). Various application cases – paints, glass coating, ceramic tiles, etc. – and several production pathways for the synthesis of this ENM have been reported in the last couple of years; however, the comparison between these various production pathways – and the consequences on the results of an application case – can be found so far in the literature. Similar situation concerning the assessment of releases of nanoparticles of TiO₂ into the environment – the recent literature reports several different approaches/procedures, but again, no comparison of these approaches can be found. Therefore the special session “LCA of nanotechnology-based products” will show the results of the currently on-going comparison of these various LCI models for the nano-TiO₂ production and of the different LCIA characterisation factors that have been established in the recent past by applying this range of LCI resp. LCA data on some of the published case studies mentioned above. References Hirschier R, Nowack B, Gotschall F, Hincapie I, Steinfeldt M, Som C (2015) Life Cycle Assessment of Façade Coating Systems containing Manufactured Nanomaterials. Journal of Nanoparticle Research 17 (68):13 pp. doi:10.1007/s11051-015-2881-0 Hirschier R, Walser T (2012) Environmental Sustainability Assessment of Engineered Nanomaterials: State of Art & Strategies to Overcome Existing Gaps. Science of the Total Environment 425:271-282 Miselec M, Olsen SI (2014) Life-cycle assessment of engineered nanomaterials: a multi-criteria assessment of functionalised nanomaterials in Europe and the world. J Nanopart Res 14 (1109):11 pp. Pini M, Cedillo González EJ, Neri P, Siligardi C, Ferrari AM Life cycle assessment of nanoTiO₂ coated self-cleaning glass. In: Technology Conference and Expo, NSTI-Nanotech 2013. pp 728-731 Pini M, Rosa R, Neri P, Bondioli F, Ferrari AM (2015) Environmental assessment of a bottom-up hydrolytic synthesis of TiO₂ nanoparticles. Green Chemistry 17 (1):518-531 Salieri B, Rigghi S, Pasteris A, Olsen SI (2015) Freshwater ecotoxicity characterisation factor for metal oxide nanoparticles: A case study on titanium dioxide nanoparticles. Science of the Total Environment 505:494-502. doi:http://dx.doi.org/10.1016/j.scitotenv.2014.09.107

576 Assessing the direct human health and ecosystem impacts of nanoparticles (or nanomaterials) in LCA O. Jollivet, University of Michigan / Environmental Health Sciences School of Public Health

577 Sustainability Check of nano-enabled products and applications C. Hidalgo, Oeko-Institut e V / Division Sustainable Products and Material Flows As part of the current debate on the opportunities and risks of nanotechnology, the need to quantify possible contributions to sustainable development is becoming increas-ingly important. Against this background, the Nano-Sustainability Check developed by Oeko-Institut provides an instrument offering an integrated approach relative to sustainability aspects of nano-enabled products and applications throughout their whole life cycle. The approach allows to serve as a strategic radar system for the management of oppor-tunities and threats, in order to anticipate beneficial effects for the environ-ment and to identify new markets on the one hand, and on the other to avoid bad invest-ments and dangers to the society. With the help of the Nano-Sustainability Check, companies that develop or produce nano-enabled products and applications can carry out a self-evaluation of their own busi-ness activities. The most im-portant factor in this context is an evaluation grid by means of which a nano-enabled product or application can be analysed by comparison with an existing reference product that has been manufactured without the use of nanomaterials. In addition, the evaluation grid is able to address any possible threats. The aspects investigated within the Nano-Sustainability Check are evaluated in the form of a total of 14 key performance indicators. The focus is on aspects of environmental and cli-mate protection, but also user benefits and life-cycle costs are considered. Complementarily, the opportunity / threat analysis takes into account external conditions such as employment effects, societal benefits and risk per-cension. The results of the individual key performance indicators are combined into a single representation. To this purpose, the “SWOT analysis” originally derived from business administra-tion is adapted as a central tool in the communication of results. Based on the SWOT matrix, recommendations for a strategic optimisation of the investigated object can finally be developed. Their goal is to maximise the potential of strengths and opportunities with regard to sustainability while minimising potential negative effects of weaknesses and threats. Besides the description of the methodological approach, also the results and conclusions from selected case studies of nano-enabled products will be presented. These cover a concrete catalyst as well as electronically dimmable windows.

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Life Cycle approaches for nanotechnology-based products
T. Lijphart, TNO

Nanotechnology often aims to improve the performance, like increase energy efficiency, reduce waste production or reduce the use of scarce resources, of product systems. Although there may be benefits of applying nanotechnology it is also associated with risks for human health or the environment. For a proper comparison of these benefits and risks one cannot focus on a single stage like production or use, only a life cycle approach can help to assess all benefits and risks. Key is that the life cycle approach encompasses all the stages, cradle-to-grave, of a product system. The assessment of the benefits and/or risks may be of a qualitative, semi-quantitative or quantitative nature. In this presentation examples of both qualitative as quantitative methods will be shown. An example for a qualitative tool is LICARA nanoSCAN (LICARA, 2014b). Using the technique of multi criteria decision analysis it presents the user with an overview of both the economic, environmental and societal benefits as well as the environmental risk and public, occupational and consumer health risks. Finally an evaluation the combined benefits and risks is presented. Substance or material flow analysis is a quantitative tool and has the potential to show the flows of a nanomaterial throughout a product system and to show the emission to the environment. The tool focusses on the risks of the e.g. nano-TiO₂ (Gottschalk, Scholz, & Nowack, 2010). The tool can be used to identify hot spots of release to the environment. Life Cycle Assessment (LCA) assesses the environmental impacts of all substances in a product system and can compare the environmental impact of the nano-based product system with the conventional one. An example is the assessment of facade coating systems (Hussein et al., 2015). This in the area of LCA of nanomaterial-based products, mapping the challenges and the gaps and how should we approach them. An important point to cover in the round table discussion is how to transfer the data/knowledge needs from the LCA experts to the experts in the related areas (material science, nanotoxicology, fate) to be able to improve/modify the existing LCA tools to be fully applicable to nanotechnology-based products. Therefore, we invite experts from other related areas to participate in the round table discussion of this session.

Ecotoxicology in tropical and polar regions (I)

579 Open discussion
S. Vázquez-Campos, Safety Sustainability

After the overview given by the talks proposed for the session the discussion will be focused on: - Nanotechnology in the areas where chemical use is increasing, but where little background data is available. There is also less knowledge on fate and effects of the chemicals in tropical areas. The Mekong delta can be mentioned here. This is a region with highly intensified paddy rice agriculture, where high amounts of chemicals are being applied. There have been studies on pesticide application in the area, but little is known about the effects on aquatic organisms living in adjacent water bodies. In this study we wanted to evaluate if the toxicity risks to fish were different between rice and rice-fish farmers with and without a background in Integrated Pest Management (IPM) and between two provinces. Risks were calculated using PRIMET, a simple model that calculates a risk quotient and a predicted environmental concentration based on a minimum of input parameters and takes into account temperature dependent physico-chemical parameters. The risk was thereafter further characterized using Species Sensitivity Distributions (SSDs) based on published toxicity test fish data (LC₅₀, acute mortality) in order to derive potentially affected fraction values for fish within the rice paddy. Pesticides with definite risk to aquatic organisms based on a worst-case assumption were alpha-cypermethrin, diazinon, fenobucarb, fipronil, permethrin, phenthoate, quinclorac and the herbicide fenoxaprop-P-ethyl. Whereas the province of Can Tho applied lower amounts of pesticides, the toxicity risks were higher for all management regimes compared to Tien Giang. The results from SSDs show that farmers cultivating fish often have lower toxicity risks in their rice fields compared to non-fish farmers. However, risks were generally high with a substantial part of the fish population being affected. This study did not evaluate toxicity of pesticide mixtures or indirect effects that would likely enhance the risks to fish. The high toxicity risks found in this study could explain why integrated rice systems have not been so successful over time. The pesticides do not only pose a threat to farmed fish but also to native aquatic species. The insecticide use in Southeast Asia has not changed much in rice-fish farming, with the same substances being used today as three decades ago. For more successful integrated rice systems, the chemical input has to be reduced and the most toxic pesticides replaced.

580 Ecological Risk Assessment (ERA) of pesticide run-off from banana plantations into the river and lagoon Madre de Dios in Costa Rica using SSD, msPAF and PERPEST models
R. Čájka, Stockholm University / Dept of Ecology Environment and Plant Sciences; D.P.J. van den Brink, Alterra and Wageningen University; C. Raepert, Universidad Nacional / IRET; L.E. Castillo, Universidad Nacional, Costa Rica / IRET; J. Gunnarsson, Stockholm University / Department of Ecology Environment and Plant Sciences DEEP

Costa Rica faces environmental problems due to high pesticide use in agriculture. In this study, ecological risks to aquatic species from pesticide residues were investigated in a river and coastal lagoon system on the Caribbean coast of Costa Rica. Three recent ERA approaches were used: SSD, multi-substance PAF (msPAF) and the PERPEST model. The results from each model are presented and compared. Analysis of pesticide residues in over 200 surface water samples at IRET, UNA, Costa Rica within the TROPICA project showed occurrence of 31 pesticides: 8 insecticides, 8 herbicides and 15 fungicides. The pesticides dinuron, ametryn, ethoprophos, epoxiconazole, diazinon, pyrimethanil, chlorpyrifos, azoxystrobin, chlorothalonil and difenoconazole were frequently detected (>10% pos. detection). Due to limitations in literature data only 12 of these pesticides were assessed in SSD using data from ≥5 species. Endpoint were LC₅₀ or EC₅₀ for sensitive species i.e. primary producers (algae, aquatic plants) for herbicides, arthropods (crustaceans, aquatic invertebrates) and fish for insecticides and all groups for fungicides. High risk was caused by the organophosphates diazinon and ethoprophos (PAF>0.05). Other pesticides posed lower risk (PAF < 0.05) but hexazinone, oxyfluorfen and chlorothalonil approached 5% species affected. The msPAF assessment was carried out at five sites using data for ≥4 species. Results show that all groups are exposed to pesticide mixtures at a high risk of acute lethal effects (msPAF>0.5). For primary producers, herbicides and fungicides outweighing insecticides. For arthropods and fish max msPAF were more similar ca 0.36 at all 4 sites downstream plantations compared to a max msPAF of 0.068 upstream.. A pattern emerged in msPAF independent of organism group: highest risk was caused by herbicides, followed by fungicides, and then insecticides. These results are unexpected as insecticides were presumed to be the greatest contributor of toxicity to arthropods and fish. This is a result of herbicides and fungicides outweighing insecticides. These results show that it is essential to study toxicity caused by mixtures in multiple exposure systems. While single substance risk assessment show fairly low risks to sensitive species, mixture toxicity assessments show that organisms in the system are exposed to mixtures that lead to a higher-than-acceptable percentage of species being affected at a 50% mortality level quite frequently.

581 Predicted fish toxicity risks of pesticides used in paddy rice fields in the Mekong delta, Vietnam, and comparison between different agro-management regimes
N. Stadlinger, Stockholm University / Ecology Environment and Plant Science; H. Berg, Orebro University; P.J. van den Brink, Alterra and Wageningen University; N. Tam, Nong Lam University; J. Gunnarsson, Stockholm University / Department of Ecology Environment and Plant Sciences DEEP

Simple risk-assessment procedures are becoming increasingly important in tropical areas where chemical use is increasing, but where little background data is available. There is also less knowledge on fate and effects of the chemicals in tropical areas. The Mekong delta can be mentioned here. This is a region with highly intensified paddy rice agriculture, where high amounts of chemicals are being applied. The results from SSDs show that farmers cultivating fish often have lower toxicity risks in their rice fields compared to non-fish farmers. However, risks were generally high with a substantial part of the fish population being affected. This study did not evaluate toxicity of pesticide mixtures or indirect effects that would likely enhance the risks to fish. The high toxicity risks found in this study could explain why integrated rice systems have not been so successful over time. The pesticides do not only pose a threat to farmed fish but also to native aquatic species. The insecticide use in Southeast Asia has not changed much in rice-fish farming, with the same substances being used today as three decades ago. For more successful integrated rice systems, the chemical input has to be reduced and the most toxic pesticides replaced.
understand a battery of biological effects parameters as relevant tools for the assessment of the ecosystem health status. For that, a pilot biological effect monitoring program was launched in 3 localities of Nicaragua and 5 localities of Brazil. Fish (Oreochromis niloticus) exposed to the mixture of herbicides ametryn + tebuthiuron (control, 0.1076 and 1.076 mg L$^{-1}$, respectively in gills and liver, of tilapia ($Oreochromis niloticus$) exposed to the mixture of the herbicides ametryn and tebuthiuron (1.0, 6, respectively). These compounds are widely used in sugarcane crops, one of the predominant cultures in Brazil. Fish were exposed during 14 days to the mixture of herbicides ametryn + tebuthiuron (control, 0.1076 and 1.076 mg L$^{-1}$ active ingredient), both in the form of commercial products in concentrated suspension containing 50% a.i. Tests were conducted in duplicate by the exposure to 1/100 and 1/10 of LC$_{50}$/LC$_{96h}$, during 14 days, followed by one same period of depuration in water without the herbicides. Fish (Ls = 9.98 ± 0.56 cm; Wt = 33.48 ± 6.15 g) (n=36) were fed ad libitum and maintained in tanks of 115 L with aeration, in a density around 3.8 g L$^{-1}$, in water with temperature (26.0 ± 2 °C) and photoperiod (16:8h, light: dark) control. The concentration of MT in gill increased significantly in fish exposed to the highest concentration of AMT + TBUt mixture for seven days. After this period, the activity of this non-enzymatic antioxidant was reduced, with a more pronounced decrease in seven days of depuration (p < 0.05). The activity of CAT was decreased (p > 0.05) in fish exposed to the lowest concentration tested in 14 days of exposure. This is consistent with the gradual accumulation of products in fish tissues and the increase of exposure time. Our results indicate the negative impact of the mixture of herbicides AMT + TBUt on tilapia physiology, as well demonstrate an herbicide-induced adaptive response in this tropical species. Furthermore, these enzymatic and non-enzymatic tissue antioxidant enzymes serve as surrogate markers of exposure to oxidant pollutants in fish. These results may also be useful for the establishment of maximum herbicides concentrations limits in water bodies.

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Soil Ecotoxicology in Brazil: where we are, to go
C. C. Nigra, Embrapa Cerrados; J. C. Niemeyer, Rua Prof. Sabino Silva / Department of Life Sciences; F. M. Rodrigues da Silva Júnior, Laboratory of Pharmaceutical and Toxicological As; M. T. Nunes, Universidade de Sao Paulo / Hidraulica e Saneamento; J. Roembke, ECT Oekotoxikologie GmbH

The first publications on soil ecotoxicological tests using soils organisms in Brazil can be dated from the beginning of the 21st century as a result of the increasing consciousness of the researchers on the importance of soil compartment. The number of groups working on soil ecotoxicology has increased significantly since in Brazil, a tropical country. However, methods standardized for temperate climate may not generate realistic results in tropical conditions, since temperature, soil and ideal test organisms are different. An adaptation largely used nowadays and which turned a recommendation of standardized norms (Brazilian Association of Technical Standards - ABNT) in the country (most of them translations of ISO guidelines) is the use of the Tropical Artificial Soil - TAS, replacing the peat sphagnum for coconut fiber as source of organic matter. Another recently adopted recommendation in the norm for acute test with earthworms is the use of 25°C, instead of 20°C, although in practice, different groups all over Brazil had been using temperatures ranging from 20 to 29°C depending on the local climate. Tested substances range from herbicides, fungicides, insecticides, metals, oil, manure, sewage and others. Most studies are of prospective approach with single-species experiments trying to determine the potential impact of these substances, but nowadays stressors on ecosystems are often multi-factorial. In the case of contaminated area and higher-tier tests have also been carried out. Advances and needs regarding reference natural soils, use of relevant test species, regulatory aspects and network activities will be presented and discussed. \n
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Elevated mobility of persistent organic pollutants in the soil of a tropical rainforest
L. Nizzetto, NIVA; Q. Zhong, Chinese Academy of Sciences Guanzhou Institute of Geochemistry; X. Liu, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences / Department of Civil and Structural Engineering; K. Borga, Department of Biosciences, University of Oslo / Department of Biosciences; J. Starrett, NIVA; J. Li, Y. Jiang, X. Liu, Chinese Academy of Sciences Guanzhou Institute of Geochemistry; K.C. Jones, Lancaster University / Lancaster Environment Centre; G. Zhang, Guangzhou institute of Geochemistry Chinese Academy of Sciences / State Key Laboratory of Organic Geochemistry Semi-volatile persistent organic pollutants (POP) are bioaccumulative and toxic contaminants. Their global distribution depends on source distribution, atmospheric transport, degradation and the exchange with ocean and land surface. Forests are crucial terrestrial reservoirs due to the commonly envisaged high capacity of their surface soils to store and immobilize airborne contaminants bound to soil organic matter. Here we show instead that POPs can be unexpectedly mobile in the soil of a tropical rainforest due to fast litter degradation (leading to rapid POP transfer to the surface soil) and leaching rates exceeding degradation rates. This determines a markedly different distribution pattern with lower bulk capacity for POPs accumulation compared to soils of colder environments. Tropical forests represent 60% of the global terrestrial production expectedly playing a relevant role in POP cycling. Hence our results suggest that biogeochemistry of organic matter degradation can represent a key control on the overall contaminant fate in soil.

Multistress in aquatic environments: the big picture (IV)
R. B. Schaefer, University Koblenz Landau / Institute for Environmental Sciences; B. Kühn, Institute for Environmental Sciences University of KoblenzLandau; E. Malaj, Helmholtz Centre for Environmental Research-UFZ / Department of EffectDirected Analysis; R. Gerig, Institute for Environmental Sciences University of KoblenzLandau

Freshwater ecosystems are threatened by multiple stressors including habitat degradation, pollution and invasive species. There is a paucity of studies on the relative importance of organic toxicants for the ecological status of rivers and their co-occurrence with other stressors. We used german monitoring data to analyse the individual and joint occurrence of four stressors, namely habitat degradation, invasive species, excessive nutrients and organic pollution. All stressors were examined for potential ecological effects based on the exceedances of low and high risk thresholds, which were based on previous studies and regulatory thresholds. At approximately 85% of the sites nutrients and habitat degradation exceeded ecological thresholds, whereas in approximately 50% of the sites the thresholds for invasive species and organic toxicants were exceeded. All sites, where only one or more stressors were available, displayed threshold exceedance from at least one stessor. Toxicity was weakly positively correlated with nutrients and habitat degradation. The risks of ecological effects from habitat degradation and invasive species were higher in lowland streams, which was particularly pronounced for invasive species. Our assessment demonstrates that habitat degradation and nutrients are dominant stressors, although we likely underestimated the risk from organic toxicants and invasive species. All sites were at risk from multiple stressors and mitigation focusing on individual stressors is unlikely to improve the ecological status. The risk of ecological effects from organic toxicants is prevalent and previous studies showed that organic toxicants may interact with other stressors in complex ways. Hence, integrating freshwater ecology and ecotoxicology is pivotal to tackle the challenge of multiple stressors.

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Modelling combined effects of exposure to metals and global warming: from individual energy budgets to ecosystem processing
N. Galis, School of Biological Sciences / School of Biological Sciences; V.E.
Forbes, University of Nebraska Lincoln / School of Biological Sciences
Freshwater ecosystems provide a number of services that are essential for human well-being, but are, at the same time, jeopardized by a range of anthropogenic activities. Changes in historical temperature regimes are exacerbated by changes in global temperatures, predicted to rise anywhere between 0.5° and 4° C by the end of the century, which is already impacting ecosystems worldwide. All these changes are associated with potential effects on biotic interactions, but also on chemical toxicity. Temperature-dependence of chemical toxicity is an emerging topic of research, but has until now been focused mainly on deriving temperature-dependent ECx or assessment factors. We here present a study that integrates temperature driven sublethal effects of metals - cadmium and copper - on energy budgets of freshwater amphipods in a simulated environment where the temperature and temperature regime can be varied over long time periods. We model the individual life histories, population dynamics and ecosystem processing. To this end, we developed an individual-based model (IBM) of a freshwater amphipod, Gammarus pseudolimnaeus. This amphipod is a shrimpfish of leaf litter, and thus plays an important role in the decomposition process in freshwater ecosystems. Individual life histories are based on energy budget dynamics which are driven by external temperature, resource availability and anthropogenic contaminants. The model has been parameterized on lab and field data. We simulated a number of temperature and toxicity scenarios and compared the results to baseline simulations. Exposure profiles were assumed to be constant and at sublethal concentrations. The metal toxicity submodel was implemented as a function of temperature in two ways: -hump-shaped where toxicity increases below and above an optimal temperature, and -linear where toxicity steadily increases with temperature. In addition, we also modeled temperature independent metal toxicity. We show how combining independent and interactive effects of metal toxicity and warming conditions yields different predictions about possible effects on different levels - individual, population and ecosystem. The extent of these effects depended on the temperature-dependent shape of chemical toxicity and on the relative thermal changes. With our modelling approach, we are able to identify systems and scenarios potentially most at risk.

589 Multistress assessment in a Mediterranean River: a field study
M. Barrera, I. Rizzardi, University of Parma / Hydraulics and Sanitation; N. Colín, A. Maceda- Veiga, University of Barcelona / Department of Animal Biology; B. Wassnurn, University of Gothenburg / Department of Biological and Environmental Sciences; J. Sturtevant, Goteborg University / Zoophysiology Dept; D. Fernandes, Institute of Environmental Assessment and Water Research IDAEA-CSIC; C. Porte, CSIC / IQAB / Environmental Chemistry; The Ripoll River is a small Mediterranean river strongly affected by human pressure. Over the summer period, urban and industrial discharges arrive into the river with little dilution. In order to assess the water quality during this period, barbels -Barbus meridionalis- and catalan chubs -Squalius laisitans-, were collected from six sites, including stressed areas receiving urban and industrial waste water along the city of Sabadell, and potential reference sites, upstream the river. Hydroxylated PAHs, nonylphenol, octylphenol and galaxolide were analysed in bile as a measure of recent exposure to these pollutants in conjunction with different biochemical responses including 7-ethoxyresorufin-O-deethylase (EROD) and 7-benzyloxy-4-trifluoromethyl-coumarin O-debenzyloxyase (BFCOD). Additionally, CYP19 aromatase activity was determined in the ovaries of female barbels as a potential marker of estrogen disruption. Levels of the aromatase activity were chemically higher in the stressed site compared to the reference sites, which indicate that fish from the lower course of the Ripoll River are strongly impacted by anthropogenic activities. Accordingly, a significant induction of EROD (9 to 10 fold) and BFCOD (3 to 5 fold) activities were determined in both barbels and chubs. Increased CYP19 aromatase activity was detected in females collected in the stressed sites in comparison to those from reference areas. Overall, this study highlights the impact of urban and industrial effluents in common Mediterranean species from the lower course of the Ripoll River. Keywords: Biomarkers, fish, Mediterranean rivers, pollution

589 Understanding salinity and acid mine drainage as stressors of Cardinia nilotica
Salinisation of freshwater ecosystems is recognised as a global major environmental challenge. In South Africa, freshwater bodies face two-fold interwined problems: increased salinity and acid mine drainage (AMD). For over a century the country’s freshwater bodies have been receiving large amounts of AMD and salts. These problems potentially threaten the country’s freshwater species including macroinvertebrates such as indigenous Cardinia nilotica. The mining industry is considered as one of the economic backbones for South Africa. However, coal and gold mining have been widely identified as the major culprits of AMD release which is associated with high sulphate and increased heavy metal concentrations. During coal mining, the rock pyrite gets fractured, oxygenated and produces sulphuric acid which makes the water highly acidic (low pH) for many aquatic species to tolerate as run off occurs. In addition, South Africa has plenty of abandoned mining sites that decant AMD water. AMD has sharply emerged as a topical subject in recent years. Most reports in South Africa acknowledge that AMD poses negative impacts in rivers. However, there is insufficient evidence to support the risks or damages of AMD on macroinvertebrates. Although there is a recommendable ongoing rivers monitoring program by the Department of Water Affairs and Sanitation, there is lack of a clear trend at spatial and temporal scales that quantifies and defines AMD in various catchment systems in the country. This may be due to the lack of sampling and monitoring in many of these systems. Therefore, this study is aimed at understanding the interaction of salinity and acid mine drainage as stressors of freshwater macroinvertebrates using Cardinia nilotica, a common freshwater shrimp indigenous to Southern African rivers. Using chemical information and various toxicity tests, the study also seeks to establish both spatial and temporal trends of AMD occurrence in selected monitoring points in the Olifants River catchment system of Mpumalanga to institute the connection between AMD and salinity as stressors of freshwater macroinvertebrates. The findings are useful in policy formulations for water management and guidelines in the protection of freshwater macroinvertebrates to these combined stresses. Keywords: Acid mine drainage, Cardinia nilotica, salinity, stressors

590 Effects of pharmaceuticals on larvae and juveniles of Palaemon varians under daily variation of temperature
E. Gonzalez-Ortegon, J. Blasco, Inst. Ciencias Marinas de Andalucia / Instituto de Desarrollo en el Mar, and de Andalucia; L. LeVay, L. Gimenez, School of Ocean Sciences Bangor University
The effects of pharmaceuticals are usually conducted at non-target species, evaluating a single compound at a time and at fixed environmental conditions; in addition most studies focus on one phase of the life cycle. Although the effects of emerging compounds may not significantly affect larval stages, larval experience may influence juvenile performance and the likelihood of successful recruitment to the adult population. The objective of this study was to evaluate the effect of single vs mixture toxicity of two pharmaceuticals, Sodium dichlorfenac (DS) and Cloribric Acid (CA), on larval and juvenile stages of the non-target saltmarsh species Palaemon varians. Experiments also consider the effect of pharmaceuticals and daily variation in temperature with another pharmaceutical model, clothaxazole (CLZ), in order to test whether this compound has similar toxic effect than on Palaemon serratus larvae. We considered cyclic variations in temperature because larvae develop commonly in saltmarshes where water temperature may vary considerably at the daily scale. The individual exposure of DS and CA at environmental concentrations (10 and 20 µg/L) did not have any effect on survival and duration of development. The effects of these PhCxs on larvae appeared more with the mixture of DS and CA at intermediate doses (234 and 194 µg/L): this mixture increased the duration of development and immoutal period required to reach the first juvenile stage. The mixture also reduced growth rates at 18°C leading to a reduction in body mass. At daily variation of temperature, the mixture of the three compounds (DS, CA and CLZ) at intermediate doses increased duration of development, decreased growth rate and larval body mass under daily variation of temperature. Some effects of pharmaceuticals appeared later during juvenile phase. This study manifested the importance to test environmental risk assessment taking into account species specific habitat conditions, the mixture of different emerging compounds and stage on larval and juvenile development and the likely complex life cycle. Acknowledgement - Financial support to EGO was given by Marie Curie fellowship and SCARCE (Consolider-Ingenio 2010 CSD2009-00065).

591 Linking multi-pollutant stress to adverse outcomes
P. Antczak, University of Liverpool / Institute of Integrative Biology; T. Williams, University of Birmingham / School of Biosciences; M. Sebire, J. Elphinstone-Davis, Cefas; M.R. Viant, University of Birmingham / School of Biosciences; J. Chipman, The University of Birmingham / School of Biosciences; I. Katsiadaki, Cefas / Environment and Animal Health; F. Falciani, University of Liverpool / Functional Genomics and Evolutionary Biology
Understanding the effect of multiple stressors on aquatic organisms is vital for environmental monitoring and risk assessment. Stressors within these environmental compartments encompass perturbations in environmental factors, such as temperature, pH or oxygen saturation, and anthropogenic contaminants. Pollutants in particular are of major concern as their effect, specifically in mixtures, is largely unknown. Assessing the effects of these stressors has proven to be a challenging task due to the observation of non-additive effects which may have devastating outcomes in local aquatic populations. This is only aggravated by the scarcity of toxicity information relating to chemicals shown to be present in the environment. To address these limitations, ‘omics’ technology has been utilized to provide an unbiased approach to measure effects of compounds and to show that the molecular state of organisms can be predictive of exposure. While these successful applications of molecular toxicity identification evaluation (mTIE) approaches have shown great potential, they generally focus on acute transcriptional response and do not provide predictive ability of chronic endpoints. Here we present a computational framework that addresses these limitations by modelling...
non-additive mixture effects from acute transcriptional responses and linking those to chronic outcomes. The nature of our approach allows us to develop putative adverse outcome pathways (AOPs) which provide putative molecular initiating events (pMIE) linked through key events (KE), as observed via perturbation of pathway activity, through to measured chronic endpoints within our experimental system. We show that our approach successfully identifies key pathway perturbations linked to chronic endpoints which can be predicted via gene expression profiles derived from single chemical exposures.

Challenges and Opportunities in Protecting Human and Ecosystem Health: Understanding the Fate, Transport, and Toxicity of Wastewater-borne Contaminants (I)

592 A novel exposure model for chemicals used globally in HPC products: A Case study and evaluation
J.E. Hodges, Unilever / SEAC; J. Kilgallon, Unilever; O.R. Price, Unilever / Colworth Science Park; A. Franco, Unilever / Safety and Environmental Assurance Centre; R. Yamshi, C.M. Holmes, V. Schlater, Waterborne Environmental Inc. The global use of a range of home and personal care products is increasing. Industry has a responsibility to assess the environmental safety of chemicals used in consumer goods in all markets, not only in regions where regulations exist. Using spatially explicit data sets we present a global model, the Scenario Assembly Tool (ScenAT) to predict in-river concentrations of chemicals used in home and personal care (HPC) products. A case study has been performed estimating Predicted Environmental Concentration (PEC) for three perfluoroalkyl substances (PFOA) calculated using a European per capita use value. Regional and country profiles of important variables (e.g. water use and sanitation practice, receiving water dilution) are explored and the impact of these variables on emission estimates and PEC distributions in major regions around the globe are illustrated and discussed. The PEC estimates for LAS are likely to be a conservative (over) estimate especially for developing countries and the globe as a European per capita usage estimate has been applied to all countries. In areas where higher PECs are forecast, further understanding into disposal practices and how products are used can be developed. Distributions will be shown for PECs, water use, dilution factors and sewage treatment connectivity for selected key countries. A preliminary evaluation exercise has been performed in China modeling the chemical ingredient Triclosan in ScenAT. On Comparison of measured concentrations and modelled urban concentrations, PECs were slightly higher than measured data (by a factor of 1.2 to 6.0). Further evaluation has been undertaken in the United States. Based on the water quality monitoring data sets available, 5 chemicals were run through the ScenAT model. The dataset showed good geographical coverage of the US and included temporal data, therefore a methodology to compare point monitoring data with polygons (ScenAT results) was created. For the majority of chemicals analysed good correlations were found between modelled and observed data. The applicability of the proposed model evaluation methodology is discussed in relation to spatial and temporal resolution of datasets. It is possible to generate improved PEC estimates at a refined scale using screening level models. This allows identification of geographic locations for further investigation or more refined exposure modeling and can inform targeted monitoring campaigns.

593 A quantitative ranking model to evaluate the environmental fate of “classic” and emerging contaminants in biosolids amended land and potential transport to drinking water
r.M. Clarke, University College Dublin / Biosystems Engineering; E. Cummins, University College Dublin / Biosystems Engineering. There have been concerns in recent years regarding the safety of biosolids as organic and inorganic contaminants remain in biosolids (post wastewater treatment), and can potentially infiltrate the environment following land application. The primary objective of this study was to develop a quantitative human health risk assessment model for “classic” and emerging contaminants in biosolids in Ireland. Sixteen “classic” and emerging contaminants, including surfactants (nonylphenol (NP), its short ethoxy chain precursors nonylphenol mono-(NP1EO), and di-ethoxy (NP2EO)), perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) substances, PPCPs (triclosan, triclocarban, carbamazepine), PBDEs (nonylphenol (NP1EO), and sterols (estriol and estradiol) were ranked to identify those that may pose the greatest risk to surface and drinking water and ultimately human health. A probabilistic risk assessment model was developed based on existing knowledge regarding contaminant behaviour and flow. The model was based on a comparison of the Predicted Environmental Concentration of contaminants in surface runoff (PEC runoff) to the No Observed Adverse Effects Level (NOAEL), resulting in a chemical toxicity impact Risk Statistic (RR). The model used probability density distributions to characterise the uncertainty and variability in input parameters while the outputs were simulated using Monte Carlo techniques. The results of the PEC runoff indicate that from the 16 contaminants analysed, the highest PEC runoff value obtained was from triclocarban with a mean PEC runoff value of 3.9E+04 µg/L. The human exposure risk ranking model identified the top 5 contaminants that can be considered as priority contaminants requiring vigilance in Ireland, these were: PFOS, PFOA, estradiol, triclocarban and BPA (with adult risk ratio values of 3.1E-01, 7.6E-02, 1.8E-02, 1.1E-02, 8.2E-03, respectively. A sensitivity analysis of model inputs revealed that the soil sorption coefficient (Koc) and the soil organic carbon (SOC) were the most important parameters that affected the variance in the model predictions (Correlation coefficient values -0.61 and -0.57, respectively). The model developed in this study is of importance to risk managers in providing a ranking of potential chemical hazards resulting from the spreading of biosolids on agricultural land, while highlighting some emerging contaminants requiring vigilance in the future.

Sustainability and Precare Allocatory Principle in Source Water Protection For Drinking Water Production
I. Brüning, IAWR; A. Bannink, RIWA
From the seventies of last century the waterworks along the Rhine river in Europe (International Association of Waterworks in the Rhine basin, IAWR) have been active in the improvement of the Rhine water quality. The ultimate goal has always been a source water quality that allows the production of perfect drinking water using simple treatment only. Already in 1973 IAWR published a Rhine Memorandum stating quality demands that would help reach that goal. Interestingly, those very demands were subsequently used by the European Union as the basis of a Directive on the abstraction of surface water for drinking water production (75/440/EC). Several updates of this memorandum were published over the years. In 2013 other associations of water works also endorsed the underlying philosophy. In 2010 the EU Water Framework Directive (WFD) was approved as EU law, including the Elbe-, and Rhine Memorandum -European rivers which provide ground filtered water for drinking water production- representing over 170 utilities in 17 European countries, with well over 115 million consumers. The most important demands for a Source Water Protection are quality objectives for not otherwise regulated variables as groups, and demands and suggestions to (political) decision makers on the achievement of the desired water quality based on the precautionary approach.

595 Comparison of the impact of two WWTPs discharges on PAHs in suspended particulate matter (SPM) and sediment contamination in chalk streams A. Chiffri, Laboratoire Chrono Environnement / Department of ChronoEnvironment; A. Bolard, E. Chanez, F. Degiorghi, University of FrancheComte / Department of ChronoEnvironment; P. Badot, University of FrancheComte CNRS / Laboratoire Chronoenvironnement Since 2009, several chalk streams of Northeast France, has been affected by repeated increased fish mortality and loss of macro-invertebrate density and diversity. Wastewater treatment plants (WWTPs) are considered as a major source of organic pollutants in freshwater ecosystems and could participate to the degradation of the river quality. Indeed, WWTP generally receive complex mixtures containing a wide variety of organic pollutants from domestic, industrial and storm-water sources. So they represented an important source of organic contaminants such as metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) and in the environment. The model was based on the Rhine Memorandum for PAHs and PCBs that were measured in river water and in sediments. A sensitivity analysis of model inputs revealed that the soil properties (e.g. water use and sanitation practice, receiving water dilution) were the most important parameters that affected the variance in the model predictions (Correlation coefficient values -0.61 and -0.57, respectively). The model developed in this study is of importance to risk managers in providing a ranking of potential chemical hazards resulting from the spreading of biosolids on agricultural land, while highlighting some emerging contaminants requiring vigilance in the future.
various conditions (natural versus purified waters, pH using simulated sunlight, and the tendency of these photo-TPs to undergo biodegradation by sediment bacteria. The phototransformation half-life of SMX ranged from 5.2 - 32.5 hours and increased with pH. SMX tended to be more persistent in natural waters compared to acidic purified waters, likely due to a combination of radical scavenging (e.g., by nitrate), light absorption (e.g., by natural organic matters), and protonation state. Some of the seven phototransformation products did not undergo further photolysis. Remarkably, these same TP s, some of which have antibacterial properties, were also found to be recalcitrant during sediment biodegradation experiments. Also notable was an increase in SMX concentrations during biodegradation experiments, possibly indicating reformulation of the parent compound from its photo-TPs by sediment bacteria. While this finding remains tentative at this time, such a process could contribute significantly to the persistence of SMX in the environment. These data highlight the importance of considering multiple transformation processes and products during the risk assessment of emerging organic contaminants.

597 Detection of physiological activities of pharmaceuticals in river water in Japan M. Ibara, H. Zhang, S. Hanamoto, H. Tanaka, Kyoto University

Over recent years, growing numbers of human pharmaceuticals have been detected in effluents of wastewater treatment plants (WWTPs) and river water. Although these are generally found at very low levels (e.g., ng/L to μg/L) in these samples, concern about their potential risks to aquatic species has been raised because they are designed to be biologically active. Before we can determine whether pharmaceuticals in aquatic environments are a threat to aquatic organisms, we must know (1) which physiological activities, determined by mode of action (MoA), are detectable in environmental water, and, if so, (2) the extent to which aquatic organisms might be exposed to these activities. Membrane receptors act like an inbox for messages in the form of peptides, lipids, and proteins. G protein-coupled receptors (GPCRs) are the largest group of these cell surface receptors in eukaryotes, and participate in various physiological and pathophysiological processes. It is estimated that nearly half of all marketed pharmaceuticals act by binding to GPCRs: for example, antihypertensives, antipsychotics, antidepressants, antiallergics, and antinasthmatics. In 2012, Iroue et al. developed an in vitro transmembrane growth factor-α (TGF-α) shedding assay, in which GPCR activation is measured as ectodomain shedding of a membrane-bound preform of alkaline phosphatase-tagged TGFα (AP-TGFα) by TNFα-converting enzyme (TACE) and its release into conditioned medium. The TGFα shedding assay can detect not only activation but also inhibition of GPCRs via all four Gα subunits, and is a very simple and rapid tool. In this study, we investigated the physiological activities of pharmaceuticals in a WWTP effluent in Japan, and upstream and downstream of the WWTP using the TGFα shedding assay. We selected receptors for angiotensin (AT1), dopamine (D2), acetylcholine (M1), histamine (H1), and so on, and investigated (1) whether GPCR was activated or inhibited by wastewater and river water extracts, and (2) whether the WWTP effluent affect the physiological activities of pharmaceuticals in river water.

Innovations in environmental analytical chemistry: the quest for pollutants using target and non-target approaches (IV)

598 Analytical and chemometric tools for the comprehensive study of complex environmental matrices by High-Resolution Mass Spectrometry

A. KISS, Institut des Sciences Analytiques / TRACES Team; A. Gonzales, S. Baig, Degremont; E. Vulliet, CNRS

Due to recent technological advancements, the comprehensive (non-target) analysis of complex environmental matrices is now a high potential strategy characterised by robustness and high throughput. This growing strategy is now recognized as a promising analytical field for various types of environmental chemistry and human health studies. In this context, we chose to illustrate a complete workflow for the interpretation of molecular prints with a special emphasis on the contribution of high resolution mass spectrometry (HRMS). The results presented here are issued from the study of an integrated water treatment system combining biological and chemical oxidation, and the characterization of new analytical methods developed for the analysis of new antibiotics and methodologies based on comprehensive molecular prints in order to (i) identify and prioritize emerging contaminants and (ii) to contribute to the assessment of the performance. This analytical strategy was implemented on a LC-Q-ToF instrument and consisted in two main steps: (i) a target and non-target screening for compounds and their transformation (degradation) products (monoisotopic exact masses: target print, multiple monoisotopic exact masses: defect) and (ii) a comprehensive study based on the comparison of 2D molecular prints (m/z, retention time) by multivariate chemometric approaches such as Principal Component Analysis. In order to illustrate the strengths and the limitations of mass spectrometry for the annotation and identification of small molecules, we will thoroughly discuss specific notions based on practical examples obtained during this study (resolution, precision, the exception: monoisotopic mass, mass defect and isotopic profile, fragmentation). The main challenges regarding the quality of the molecular prints (i.e. acquisition and data analysis parameters) will be also emphasised during this talk. The comprehensive approach applied in this study led to information-rich molecular prints which successfully complement the previous target study by offering a broader view of the samples. The use of separation techniques coupled to high resolution mass spectrometry is fully justified by (i) the high number of detected signals, (ii) the wide dynamic range, (iii) the diversity of physicochemical properties, (iv) the presence of isomers and (v) the need for an additional dimension in order to discriminate the signals having similar m/z ratios.

599 Prediction of pharmaceutical and illicit drug residue retention times in wastewater extract data acquired by gradient liquid chromatography-high resolution mass spectrometry

I. Barren, Kings College London / Analytical and Environmental Science; K. Munro, Analytical and Environmental Science; T. Munro, Analytical and Environmental Science; C. P. Martins, A. Edge, thermo Fisher Scientific; D. A. Cowan, Kings College London / Drug Control Centre

The application of in silico tools for post-data acquisition mining of liquid chromatography-high resolution mass spectrometry (LC-HRMS) data holds potential to more rapidly identify novel or emerging pollutants. Data-mining tools are currently available for mass screening of complex HRMS data, but suspect confirmation still requires a matching chromatographic retention time (tR) with a reference standard, which are often prohibitively expensive, limited or require synthesis ab initio. Even with HRMS technology, several viable chromatographic peaks at isotopic m/z may exist in environmental extracts, which makes selection of reference materials for synthesis even more challenging. Currently, appropriate tR prediction tools for small ionisable molecules such as pharmaceuticals, personal care products and illicit drugs in complex samples are lacking. Herein, the development, characterisation and application of an accurate neural network (ANN) for prediction of gradient reversed-phase liquid chromatographic retention times (tR) for 166 pharmaceuticals in wastewater extracts is presented for the first time and coupled with qualitative HRMS data mining methods. Multiple ANN architectures including radial basis function (RBF), multilayer perceptron (MLP) and generalised regression neural network (GRNN) were investigated and a comparison of their predictive ability for model solutions discussed. The influence of wastewater matrix complexity on tR showed a variance of > 1 min for some compounds in comparison to model solutions. Similarly, matrix impact on ANN predictive ability was addressed towards developing a more robust approach for routine data screening applications. Overall, the optimised GRNN-type model had a predictive accuracy of < 1.3 min at the 75th percentile of all measured tR data in wastewater samples (2±0.92). Finally, the model was evaluated for application to the semi-targeted identification of pharmaceutical residues during a weeklong wastewater sampling campaign. The model successfully identified native compounds not included in an HRMS activity list (from the United States Pharmacopoeia list) with a rate of 82±5 % and 74±5 % in influent and effluent extracts respectively. Ultimately, this research will potentially enable faster identification of emerging contaminants in the environment through more efficient post-acquisition data mining.

600 Sensors for in-situ monitoring of Eutrophication

M. McCaul, National Centre for Sensing Research / National Centre for Sensing Research; J. Cleafy, Carlow Institute of Technology; E. McNamara, D. Diamond, Dublin City University / National Centre for Sensing Research

This work is carried out as part of the COMMON SENSE European FP7 project. The COMMON SENSE project aims to provide a reliable sensing platform for in-situ measurements on key water quality parameters relating to eutrophication, heavy metal contaminants, marine litter and underwater noise. The COMMON SENSE nutrient sensor is based on a combination of microfluidic analytical systems, colorimetric reagent chemistry, low-cost LED-based optical detection, and wireless communications. The reliable quantification of nutrients in marine environments is challenging due to the low concentration of these solutes in the ocean and the nature of the matrix in which they are held. Initial studies are focused on validating a method for the sequential determination of nitrite and nitrate in marine environments. Coupled with the traditional well established Griess–Biswas reaction for the determination of nitrite, a vanadium chloride (VCl3) solution is used as the reducing agent. The method shows potential as an alternative approach to toxic colourimetric methods for the determination of nitrate and nitrite in marine waters as results indicate that there is no apparent interference from variences in background salinity. The method was tested on a series of samples with varying salinities and sample matrices (costal, estuarine and freshwater), the method is low cost, reproducible and requires low volumes of sample and reagents.

601 Using UV LEDs to Limit Biofilm Formation with Applications for Optical Sensors

K. Murphy, Dublin City University / NSC; A. Barrett, Dublin City University / Department of Chemical Sciences; T. Sullivan, J. Moreno, Dublin City University / Department of Chemical Sciences; I. Regan, Dublin City University / Chemical Sciences

Biofouling is a serious, longstanding issue for optical detection systems working in aquatic environments. UV radiation has long been known as having a germicidal and biocidal effect, particularly in the UV-C regime. This effect has been used for applications such as water sterilisation and food safety. It has also garnered interest...
among researchers of environmental sensing as an anti-foulant, or as an aid to it, in particular for optical sensors. The increase in the volume of sensors means that the cost of ownership of sensors needs to be addressed, making biofouling abatement a key issue. Traditionally, logistical and cost reasons prevented the use of UV light on optical surfaces underwater, with broadband UV lamps being unsuitable and impractical for use with individual optical sensors. Recent advances in UV LED technology makes it technically possible to use such light for the reduction of biofouling in these types of sensors. Some investigations have been taken place of late, focusing on water disinfection, into the use UV LEDs, of distinct wavelengths, for germicidal purposes. Harnessing the germicidal and biocidal effects of UV radiation using LEDs could lead to a cost- and power-effective method for anti-fouling with in-situ sensors for aquatic environments. In this abstract we present early results of biofilm growth abatement on polymer surfaces with UV LED radiation treatment, in a laboratory environment conducive to the growth of biofilms. Commercially available, competitively priced LEDs are utilised in this work (UVTOP UV-LEDs). These LEDs have a peak wavelength of 285nm, a FWHM of 12 nm and an optical power at 20mA of 0.8mW. A water bath incubator is seeded with Chlorella Vulgaris algae and PDMS coupons are placed into it, with some illuminated by the UV LED and some unexposed as control coupons. The exposed coupon is covered and so is unaffected by ambient light. The control coupons are subject to ambient light conditions. Using this setup a number of trials were carried out, with the level of biofilm growth on the coupons quantified using epifluorescence microscopy and a cell counting image analysis program. Initial results from a test series, of one test and one control coupon each split into four quadrants, are presented here. The develop a biosensor based on immunosensor system in the eastern dem of China, where the majority of the UV is concentrated but fades away towards the edge of the coupon.

Development of an indirect enzyme-linked immunosorbent assay for the detection of sulphonamides in seawater

C. Bosch, IDAEA-CSIC; J. Salvador, P. Marco, Institut Catalá de Nanociència i Nanotecnologia ICN CSIC CIBERBBDN; M. Farre, IDAEA-CSIC; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research Aquaculture has increased and intensified during last decades all over the world. The widespread of these activities have displayed some other inconveniences in the case of the antibiotics abuse. Although these compounds are very efficient to treat bacterial diseases in the marine organisms, are also used in prophylactic therapy and for growth promotion, causing their propagation along the aquatic environment as well as along the food chain. Moreover, it has been demonstrated that antibiotics promote the bacterial acquired resistance even at low concentration levels. Sulphonamides (SAs) are a group of antibiotics, widely used in aquaculture. Seven of the most frequently used SAs have been studied in this work - sulfadiazine (SDZ), sulfathiazole (STZ), sulfapyridine (SPY), sulfadimethazine (SDZ), sulfamethoxy-pyridazine (SMP) and sulfonaxoquine (SQ). A competitive indirect enzyme-linked immunosorbent assay (ELISA) has been developed to determine the presence of these compounds in seawater. The results obtained will be used to develop a biosensor based on immunosensor system in the eastern dem of China, where the majority of the UV is concentrated but fades away towards the edge of the coupon.

603 Pesticides in seawater: method development & fate in Catalonia coastal areas (NE-Spain).

M. Kliek-Schulmeyer, Water and Soil Quality Research Group Dept of Environmental Chemistry; M. López de Alda, Institute of Environmental Assessment and Water Research IDAEA CSIC / Environmental Chemistry; M. Farre, IDAEA-CSIC; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research

Anthropogenic activities in coastal areas lead to loss of species and populations, phytobenthos degradation and community change, and introduction of non-indigenous species. Among the different pollutants, the group of endocrine disrupting chemicals, which is an extensive and heterogeneous group of substances that may interfere with the endocrine system and hormone activities of organisms, is especially relevant. Within this category, pesticides stand out for being chemicals extensively used in both agricultural and urban areas, reaching environmental waters through diverse processes. In this context, the main goals of this study were the optimization and validation of an analytical approach suitable for the assessment of multiple pesticides (more than 30, including some transformation products) in seawater, based on solid phase extraction (SPE) followed by liquid chromatography-electrospray-tandem mass spectrometry (LC-MS/MS), and its application in the analysis of different coastal seawater (40 samples) collected along the Catalan coast. This goal is under consideration in the SEA-on-a-CHIP project (which aims to develop new analytical approaches and a miniaturized, autonomous, remote operated sensor system able to analyse organic contaminants affecting our and produced by aquaculture in marine waters under multi-stressor conditions) and the Solutions project (which aims to develop tools for the identification, prioritisation and assessment of marine pollutants that may make a risk to ecosystems and human health). The method developed showed satisfactory performance in the analysis of the target pesticides in both HPLC water and seawater. The analytical method presented recovery rates (calculated after blank subtraction) between 70 and 120% relative to the corresponding deuterated standards in most cases. In addition, good sensitivity with method limits of detection (MLOD) in the ng/L range or lower was achieved in real seawater samples. Satisfactory linearity and repeatability (with relative standard deviations below 20% in most of the cases) were also obtained. In the present study, concentrations at ng/L levels of several of the target compounds were found, in agreement with the results of previously published studies. In addition, hot spots related to pesticides contamination along the Catalan coast were identified. The potential sources of contamination by pesticides are also hypothesized and discussed.

Challenges in Wastewater Treatment and Reuse and the Agricultural Use of Manures and Biosolids (III)

604 Impacts of metals on antibiotic resistance and conjugal plasmid transfer in soil bacterial communities

K.K. Brandi, University of Copenhagen / Department of Plant and Environmental Sciences; U. Klimper, Technical University of Denmark / Department of Environmental Engineering; W. Luo, University of Copenhagen / Department of Biology; J. Song, University of Copenhagen / Department of Plant and Environmental Sciences; T.A. Johnson, Michigan State University / Center for Microbial Ecology Department of Civil and Environmental Engineering; J.J. Modrzyński, University of Copenhagen / Department of Plant and Environmental Sciences; R. Libor, University of Copenhagen / Department of Biology; M.H. Nielsen, University of Copenhagen / Department of Plant and Environmental Sciences; L.H. Hansen, Aarhus University / Department of Environmental Science; J. Jensen, Aarhus University, DMU / Department of Bioscience; A. Dechesne, Technical University of Denmark / Department of Environmental Engineering; S.A. Hashesham, Michigan State University / Center for Microbial Ecology; P.E. Holm, University of Copenhagen / Department of Plant and Environmental Sciences; B. Elberling, University of Copenhagen / Center for Permafrost, J.M. Tiedje, Michigan State University / Center for Microbial Ecology Department of Civil and Environmental Engineering; O. Nybroe, University of Copenhagen; B.F. Smets, Technical University of Denmark / Department of Environmental Engineering; S.J. Sørensen, University of Copenhagen / Department of Biology Environmental selection and horizontal gene transfer constitute key processes in the environmental development and spread of antibiotic resistance. We previously demonstrated that long-term (+85 years) exposure to Cu had co-selected for antibiotic resistance in a soil receiving no known anthropogenic inputs of antibiotics. Here, we extend our previous work on metal-induced selection pressures for the expansion of the soil bacterial resistome by studying the effects of metal ions on antibiotic resistance as a likely selection mechanism in polluted soils, but such mobile genetic elements may be present in most cases. In addition, good sensitivity with method limits of detection (MLOD) in the ng/L range or lower was achieved in real seawater samples. Satisfactory linearity and repeatability (with relative standard deviations below 20% in most of the cases) were also obtained. In the present study, concentrations at ng/L levels of several of the target compounds were found, in agreement with the results of previously published studies. In addition, hot spots related to pesticides contamination along the Catalan coast were identified. The potential sources of contamination by pesticides are also hypothesized and discussed.
Silver-induced selective pressure on environmental bacterial communities is becoming increasingly relevant due to the growing use of silver nanoparticles (AgNPs) as antimicrobial agents in biomedical supplies and other commercial products. Genetic silver resistance in Bacteria is known to exist, and it is possible that horizontal gene transfer mechanisms, such as those involving intermediate to long host range conjugative plasmids (e.g. IncH and IncP groups) [1], may facilitate the transfer of resistance among bacterial community members in densely populated environments. We conducted several different studies to investigate the effects of significant selective pressure on environmental microbial communities. In one study, 5 Australian soils with a broad range of physico-chemical properties were exposed to silver for 2 weeks (2W or fresh) or 9 months (9M or aged) at concentrations ranging from 50-400 mg/kg Ag, while unspiked soils were maintained as controls. Total DNA was extracted from the soils in triplicates and polymerase chain reaction (PCR) based counting (real-time quantitative PCR) and partial sequencing (Illumina MiSeq platform based) of the 16S rRNA gene of Bacteria in 100 samples was performed for bacterial diversity studies. In a second study, 10 different soils were exposed to 9 concentrations of Ag+ ranging from 1 – 2000 mg/kg to ensure full dose-response curves could be obtained. After a two month incubation, analysis included 16S rDNA partial sequencing for phylogenetics, quantification of key genes by real-time PCR, high throughput soil respiration by MicroResp, and a suite of 8 different enzymatic activities. Silver resistance was also tested in wastewater samples from the Adelaide treatment and reuse schemes. Collectively, our results show that the effects of silver on total environmental microbial communities depend on the pre-exposed communities, and that potentially silver-resistant pathogenic strains can be found in environmental samples.

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In situ microbial resistance to sulphonamides: quantification and fate of sulphonamide consumption in the Llobregat river.
R. Gómez, Cefas; M. Costa, CSIC-IDAEA; J. Acosta, IDAEA-CSIC; S. Perez, IDAEA-CSIC / Environment and Chemistry; D. Barceló, IQAB-CSIC; Institute for Environmental Assessment and Water Research; E.O. Casamayor, CEAB-CSIC; J. Dachs, IDAEA-CSIC / Environment and Chemistry

The occurrence and spread of antibiotic-resistant bacteria (ARB) are pressing public health problems worldwide, and aquatic ecosystems are a recognized reservoir for ARB and antibiotic resistance genes (ARGs). In order to assess the role of ARB in aquatic environments, we sampled in the mouth of the Llobregat river (NE Iberian Peninsula, downstream, polluted waters) and at the mouth of the Llobregat river (NE Iberian Peninsula, downstream, polluted waters). The activity of the benthic microbial films attached to rocks (epilithic) was investigated in response to chemical concentration of antibiotic sulphonamides. The experiment lasted for approximately 6 months and involved in situ monitoring techniques. We observed a decrease of sulphonamide antibiotics concentrations within 2 days in both pristine and polluted waters, and the production of different transformation products concomitant with a steady increase of ARG in the epilithic biofilm community. However, the two ARG tested (SULF1 and SULF2) showed different patterns. Results suggest higher effects after high additions of antibiotics, although in mesocosms the communities were fully recovered after 30 days. The microbial film act as “natural treatment systems”, resulting in decreased chemical concentrations of antibiotics.

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Definition of limits of detection and quantification of antibiotic resistance genes in soils: a first step on the assessment of the impact of wastewater irrigation
S. Teixeira, Faculdade de Engenharia - Universidade do Porto / LEPABE; Laboratório de Engenharia de Processos Ambiente Biotecnologia e Energia; C. Becerra-Castro, CBQF / UCP / CBQF; Centro de Biotecnologia e Química Fina; O.C. Nunes, Faculdade de Engenharia - Universidade do Porto / LEPABE; Laboratório de Engenharia de Processos Ambiente Biotecnologia e Energia

The spread of antibiotic resistance is considered an environmental problem worldwide that represent a serious threat to public health. Wastewater is known to be an important source of resistance, and it is possible that horizontal gene transfer mechanisms, such as those involving intermediate to long host range conjugative plasmids can spread resistance to bacteria and genes into soils. However, the correct assessment of the implications of wastewater irrigation will be limited by the minimal dose of genes or bacteria cells in soils that can be detected and quantified. In this study, we aimed at assessing the PCR and qPCR detection and quantification limit of antibiotic resistance genes in an agricultural soil. With that purpose, were settled mecrocoss assays, in which an agricultural soil was soaked with a synthetic effluent inoculated with two antibiotic resistant strains from wastewater. Antibiotic resistant strains Escherichia coli A2CC14, which harbours the genes blaTEM, blaCTX-M-15, blaOXA-1, ace(6)-Ib-br and the integrase IntI-1, and Enterococcus faecalis H1E1V10, with the gene vanA, were inoculated in agricultural soil at densities ranging from approximately 10^6 to 10^7 CFU g^-1 soil. A control was prepared using the same synthetic effluent without bacteria. Serial dilutions of soil samples were plated on m-Faecal Coliforms agar and on m-Enterococcus agar for enumeration of coliforms and enterococci, respectively. Bacteria growing on these culture media were compared with originally inoculated strains based on RAPD profiling. For gene detection and quantification, total DNA was extracted from soil with PowerSoil™ DNA Isolation Kit (MO BIO) and PCR amplification was performed for each target gene. The detection sensitivity of the conventional PCR for the blaTEM and vanA genes corresponded to bacterial densities higher than 10^7 CFU g^-1 soil. These results and other conventional and qPCR estimations will be discussed, emphasizing the importance of defining the detection and quantification limits when the fate of antibiotic resistance genes in soils irrigated with wastewater is under analysis (Keywords: antibiotic resistance, soil, wastewater).Acknowledgments: This study was carried out under the scope of the COST Action ES1403 –NEREUS and financed by FCT (PEst-OE/QIB/00016/2013, PEst-C/EB/00111/2013) and FEDER through “Programa Operacional do Norte-ON2” (LEPABE/CEFET – reinforcing R&D engineering competences in Energy, Environment & Health)

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Establishing causality in ecotoxicological risk assessment of micropollutants in wastewater effluents
A. Thill, Eawag / Environmental Toxicology; R. Behra, Eawag / Department of Environmental Toxicology

Micropollutants (MPs) released into fresh waters via wastewater treatment plants (WWTPs) are recognized as a global problem contributing to the degradation of ecosystem quality. Therefore, the aim of this field study is to causally link real effects resulting from microbial community-level exposure to complex mixture of MPs present in WWTPs effluents. To achieve this goal, we combined time-integrative passive sampling technologies with the assessment of extract toxicity via bioassays; using in situ sampled periphyton. Periphyton were sampled up- and downstream of four WWTPs effluents in Switzerland and exposed to various dilutions of extracts from passive samplers that were immersed at the effluent of each site. Tolerance of communities to Chemcatcher™ passive sampler extracts was determined according to the pollution-induced community tolerance (PICT) concept by measuring the inhibition of various functional endpoints. Specific tolerance of the heterotrophic component of periphyton was measured by 14C-leucine incorporation and MicroResp™ method to measure secondary production and heterotrophic respiration, respectively. Specific tolerance of the phototrophic component of periphyton was assessed by 13C-carbonate assimilation and algal fluorescence to measure primary production and photosynthetic yield, respectively. Additionally, we examined shifts in the structure of the up- and downstream periphyton by performing a community level physiological profile (CLPP) and a DNA fingerprint analysis. Results showed that despite low concentrations of MPs detected downstream of the effluents, phototrophic and heterotrophic components of the sampled periphyton displayed increased tolerance towards these MPs. Interestingly, in comparison to heterotrophic functional responses, phototrophic community composition did not change significantly. These findings might give a first indication on the MPs composition in the effluents (dominance of herbicides or bactericides), which was confirmed by analytical identification of MPs in the passive samplers’ extracts. The observed effects were accompanied by changes in the microbial CLPP and in molecular diversity. Overall, our study highlights the sensitivity of the proposed approach to detect effects in low MPs concentrations occurring in the field and to establish a causal link between exposure and the observed ecological effects on freshwater microbial communities.

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Real-time and in situ monitoring of aquatic environments using indigenous microbial community-based biosensors
J. Monier, O. Sibourg, ENOVEO

Increasing contamination of aquatic environments by organic loads or toxic chemicals has resulted in the need for real-time monitoring tools to be used in pollution detection and risk assessment. While monitoring aquatic environments is crucial to ensure their quality, the use of sensitive and powerful tools targeting a broad range of chemical compounds is increasingly required. In aquatic environments, species richness and biodiversity vary significantly in time and space. Therefore, the use of functional endpoints can help to identify potential biodiversity changes and to detect the presence of toxic compounds. One of the most powerful tools to achieve this is the integration of passive samplers with high throughput bioassays. Typically, passive sampling technologies are designed to monitor gradients of pollutants, especially in water. Real-time monitoring has been developed and is increasingly used by aquatic biologists to monitor water quality, environmental exposure and the occurrence of pollution events. However, while these tools are currently used to assess water quality, they are not equipped to assess the impact of pollutants on the microbial communities. This drawback is detrimental, since the microbial community can be used as a proxy of the water quality and aquatic environment. In fact, microbial communities thus have been used to monitor realistic low (eg, sewage networks, wastewater treatment plants, groundwaters) or detect toxic compounds and assess their impact on their environmental sink (eg, industrial processes and wastewater treatment plants...).
Emerging Contaminants in the Marine Environment: Presence, Effects, Regulation (I)

610 Marine monitoring campaign in France and French overseas departments: more than 10,000 datasets from grab and passive sampling

F. Botta, INERIS. A. Abarron, G. Bocquené, M. CHAMPIN, G. Durand, J. Gonzalez, J. Masson, P. Priou, C. Tixier, M. LeMoigne, IFEREMER, N. Tapie, ISCMR-LPTC - UMR CNRS; J. Lestran, S. ANDRES, INERIS; M. Dévier, UMR CNRS EPOC Equipe LPTC-EPOC, University of Bordeaux / Oceanic and Continental Environments; P. Labadie, University of Bordeaux / UMR EPOC Equipe LPTC, V. Duval, INERIS

A screening study of emerging contaminants was carried out in 2012 in coastal waters in both metropolitan France and overseas departments (Martinique, Guadeloupe, Réunion, Mayotte and French Guiana) as part of the National Action Plan against pollution of the aquatic environment, which requires the regular updating of the lists of substances to be included in monitoring programmes. The Action Plan stresses the need to set up a watch list of substances to be investigated at the national level in order to acquire missing information about the level of exposure to emerging contaminants in the aquatic environment and allow identification of substances for which specific actions need to be implemented. The Ministry of Ecology appointed ONEMA as principal contractor and INERIS as project leader. One of the main objectives of this screening study was to gain knowledge of the occurrence of substances of “emerging” concern in the marine environment. Different data were collected, such as the presence/absence of each investigated compound and the level of concentration observed in the aquatic environment (water and sediment). Moreover, the performance of different sampling techniques was assessed. This study contributes to increased knowledge of emerging pollutants in French waters and to the wider considerations for updating the list of relevant substances to be included in the new monitoring programme. It is to be established. Triple sampling of water at the same site followed by BSBE extraction displayed a high results variability, which limits the exploitation of the quantified results. Nevertheless, the testing of in situ systems integrators – POCIS – and extraction by BSBE bars has proven the added value of these two complementary techniques for monitoring hydrophobic and hydrophilic compounds in marine water. Finally, analyses of sediment allowed a more complete assessment of the quality of the waterbodies.

611 Marine monitoring shows emerging long-chained perfluorinated compounds in biota, following the ban of PFOS.

M. Hauser, Uni Research, SAM-Marin / Uni Research Environment; K.S. Hatlen, Uni Research, SAM-Marin / Environment

Perfluorinated Alkylated PFAS (PFAS) containing perfluorooctyl sulphonate (PFOS) and perfluoroctanoic acid (PFOA) were used in firefighting areas, as isomers, in the construction and manufacturing industry, and as electronic, medical, and health care products. The major use of PFAS is as flame retardants. Perfluorooctane sulfonate (PFOS); Perfluorooctanoic acid (PFOA); Perfluorooctane sulfonyl fluoride (PFOSF).

The composition profiles and cation exchange of the octane-water partition coefficients (KOW) in logarithm for compounds in PAE group, while that in other two groups didn't own a positive correlation with the octane-water partition coefficients (Kow) in logarithm for compounds in PFAE group. while that in other two groups didn't own a positive correlation with the octane-water partition coefficients (Kow) in logarithm for compounds in PFC group.

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612 Occurrence and fate of endogenous steroid hormones, alkylphenol ethoxylates, bisphenol A and phthalates in Pearl River Delta, China

Q. Wu, C. Lam, X. Zhang, City University of Hong Kong; X. Guo, Shenzhen Oceanography and Fishery Environment Monitoring Station; K. Lam, City University of Hong Kong

The endocrine disrupting chemicals (EDCs) have attracted strong scientific interest for causing hazard effect on aquatic organisms. The occurrence, distribution and potential risk of highly concern alkylphenol ethoxylates (APEOs), bisphenol A (BPA) and phthalic acid esters (PAEs) in Pearl River Delta (PRD), the “world factory”, were investigated in this study. Natural estrogens, androgen, androgen, found with higher concentration level and detect frequency, while the synthetic estrogen 17α-ethynylstradiol, Diethylstilbestrol and equilibrium were sporadically detected. For progesterin and androgen, norethindrone, progesterone and testosterone were detected at low concentration levels. Nonynylphenol-ethoxylates (n=0-2) were significantly higher than 4-tert-octyphenol-n-ethoxyllate (n=0-2), but still lower than the level from other countries. Bisphenol A concentrations were almost within the same order of magnitude from these studies all over the world, suggesting their gradually converged worldwide usage. Dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DNBP), di,iso-butyl phthalate (DIBP), bis(2-ethylhexyl) phthalate (DEHP) were the most frequently detected PAEs, but with obvious spatial distribution diversity. The concentrations of DMP in river water were significant higher than that in Shenzhen coast area, nevertheless, the concentrations of DIBP in coast seawater was predominated and were much higher than that in river samples. The composition profiles and concentration levels of the target compounds between the surface and bottom water in the same sampling location were similar, which indicated the rapid vertical diffusion. The average distribution coefficient (Kow) showed positive correlation with the octane-water partition coefficients (Kow) in logarithm for compounds in PAE group, while that in other two groups didn't own a positive correlation. A preliminary risk assessment was quantitated by risk quotient (RQ). The result showed that APEOs and PAEs had less potential to cause adverse effect to the local species (RQ<0.1), however the steroid hormones, E2 and E2E2, had higher risk for causing estrogenic effects to the marine organisms at several sampling points in the water environment (RQ>1). Therefore, it is necessary to give high priority in further reducing the level of steroid hormone in PRD region in consider of their high estrogenic activity.

613 Field Validation of an Integrative Passive Sampler for Munitions Constituents in Marine Environments

G.H. Rosen, SPAWAR Systems Center / Energy and Environmental Sustainability; G.R. Lotufo, U.S. Army ERDC / Environmental Laboratory; J.B. Belden, Oklahoma State University / Department of Zoology; R.D. George, US Navy, Spawar Systems Center 2361; V.J. Kirtay, SPAWAR SYSCECN SAN DIEGO / Energy and Environmental Sustainability; B. Wild, SPAWAR Systems Center

The measurement of trace level polar organic compounds in environmental matrices represents a significant challenge. Recent improvements in analytical techniques coupled with the development of passive sampling devices (PSDs) have much to offer towards in situ monitoring of ultra-low concentrations of legacy and emerging contaminants by providing time-integrated samples, including use of innovative PSDs. We expanded the use of POCIS to include energetic materials such as trinitrotoluene (TNT), degradation products of TNT (e.g. aminodinitrotoluenes [ADNTs] and diaminonitrotoluenes [DANTs]), and trihydroxyhexadiohydrotriazine (RDX), due to their prevalence in military munitions worldwide, and regulatory and public concern associated with contamination in the marine environment. Chemical Integrative Samplers (POCIS). We expanded the use of POCIS to include energetic materials such as trinitrotoluene (TNT), degradation products of TNT (e.g. aminodinitrotoluenes [ADNTs] and diaminonitrotoluenes [DANTs]), and trihydroxyhexadiohydrotriazine (RDX), due to their prevalence in military munitions worldwide, and regulatory and public concern associated with contamination in the marine environment. The composition profiles and cation exchange of the octane-water partition coefficients (KOW) in logarithm for compounds in PAE group, while that in other two groups didn't own a positive correlation with the octane-water partition coefficients (Kow) in logarithm for compounds in PFAE group. while that in other two groups didn't own a positive correlation with the octane-water partition coefficients (Kow) in logarithm for compounds in PFC group.

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The composition profiles and cation exchange of the octane-water partition coefficients (KOW) in logarithm for compounds in PAE group, while that in other two groups didn't own a positive correlation with the octane-water partition coefficients (Kow) in logarithm for compounds in PFAE group. while that in other two groups didn't own a positive correlation with the octane-water partition coefficients (Kow) in logarithm for compounds in PFC group.
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164 SIMULTANEOUS DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN LIVER AND MUSSEL SAMPLES

J. Zabaleta, E. Bizkarguenaga, Analytical Chemistry; A. Vallejo, M. Ortiz, L. Fernández, University of the Basque country UPV/EHU; A. Prieto, University of the Basque country UPV/EHU / Analytical Chemistry; O. Zuloaga, University of the Basque country UPV/EHU.

An analytical method for the simultaneous determination of 14 perfluorinated compounds (PFCs), including 3 perfluoroolalkylsulfonates (PFASs), 7 perfluorocarboxylic acids (PFCAs), 3 perfluorophosphonic acids (PFPPs) and perfluorooctanesulfonamide (PFOSA), and 10 precursors, including 4 polyfluoralkyl phosphates (PAPs), 4 fluorotelomer saturated acids (FTCAs) and 2 fluorotelomer unsaturated acids (FTUCAs) was developed in fish liver and mussel samples. After setting different variables affecting the chromatographic separation (column type and mobile phase), the electrospray ionization (capillary voltage, nebulizer pressure, drying gas temperature and drying gas flow) and mass spectrometric detection (fragmentor voltage and collision energy) of the target analytes, different clean-up strategies by means of solid phase extraction (SPE) using WAX, Envi-Carb or a combination of them were optimized and evaluated for the clean-up of focused ultrasonic solid-liquid (FUSLE) extracts. In all the cases the analysis was performed by liquid-chromatography-triple quadrupole mass spectrometry (LC-MS/MS). WAX coupled in line to Envi-Carb was selected and validated in terms of recovery (external calibration using isotopically labeled analogues as surrogates and matrix-matched calibration approaches were tested) and method detection limits (MDLs). Recovery values in the 65-116 % and 91-119 % range and MDLs in the 0.1-2.7 ng/g and 0.1-3.8 ng/g range were obtained for liver and mussel samples, respectively. The developed method was applied to the analysis of grey mullet liver (Chelon labrosus) and mussel (Mytilus galloprovincialis) samples from the Basque Coast (North of Spain). In the case of liver samples perfluorooctane sulfonate (PFOS), PFOSA and perfluorooctanoic acid (PFDA) were detected. The highest average PFOS concentrations were observed in Gernika (988 ng/g) followed by Deba-Mutriku (54 ng/g), Pasaia (35 ng/g), and Plentzia (26 ng/g). PFOSA and PFDA concentration were in the 4-15 ng/g and 1-2 ng/g range, respectively. In the case of mussels, although low concentration of PFOS (1.4-2.0 ng/g) and PFOSA (3.8-4.2 ng/g) were detected, 8:2 fluorotelomer phosphoric acid (FOPAP) in the range of 21-48 ng/g was detected in Vigo, Pasaia, Santurtzi, Getxo and Muskiz. To our knowledge this is the first work were 24 per- and polyfluorinated compounds are determined in fish liver and mussel and the first time that the 8:2 monoPAP has been detected in mussels.

165 Chemical Adsorption of Hydrophobic Persistent Organic Pollutants in the Marine Environment onto Microplastic Polymers and Subsequent Trophic Transfer

C. Crawford, C. Ewins, University of the West of Scotland / Institute of Biomedical and Environmental Health Research; B. Quinn, Irish Centre for Environmental Technology (ICET) / Marine and Environmental Health Research; M. Russell, Marine Scotland Science / Marine Environmental Assessment

With an approximate degradation time of 450 years, plastic is one of the most durable and persistent environmental contaminants in the modern world. Production since the 1950s has increased prodigiously. In the marine environment, photo-oxygenative degradation and the abrasive action of waves progressively degrade larger pieces of plastic into tiny polymer particles less than 5 millimetres in diameter, termed microplastics. Consequently, microplastics are inadvertently ingested by ocean dwelling biota, particularly susceptible filter feeders such as Mytilus edulis. Crucially, these organisms are located at the bottom of the food chain and recent research has demonstrated detrimental effects such as inhibition of gastrointestinal function and feeding impairment. However, it has newly emerged that microplastics are able to act as a transporter for the ups of hydrophobic persistent organic pollutants (POPs) whereby hydrophobic organic chemicals (HOCs) become adsorbed onto the surface of the microplastic particles. Compounds such as polychlorinated biphenyls, which exhibit a broad spectrum of aerobic and anaerobic bacteria, are particularly ubiquitous. Furthermore, increased chlorine substitution results in a decrease in susceptibility to photodecomposition by ultraviolet light and thus, resistance to degradation in the marine environment. Ingestion by biota, combined with the typical pH changes associated with digestion results in POPs leaching off the surface of the microplastics with the consequence of bioaccumulation within the organism. Since susceptible organisms are located at the bottom of the food chain, chemical adsorption provides a mechanism for trophic transfer up the food web to larger organisms, including humans. Consequently, chemical adsorption of a specifically nominated POP onto a polycarbonate microplastic polymer organic phase was investigated to determine the sorption capacity of the microplastic in order to evaluate the level of contaminant capable of being transported. Furthermore, subsequent submersion of the microplastic in an acidic pH environment at gut temperature enabled simulation of physiological conditions while facilitating investigation and quantification as to the level of POP leached into the surrounding medium. Consequently, this allowed determination as to the extent of leaching exhibited by POP coated polyethylene microplastics when exposed to a physiologically simulated chemical environment and the associated risk to marine biota.

166 The Evolution of Risk Assessments of Manufactured Nanomaterials over the past 10 years - A regulatory perspective

Y. Sullivan; T. Francis, B. Fisher, Environment Canada

Regulatory bodies around the world continue to assess and manage potential risks associated with manufactured nanomaterials. Over the past decade we have seen a shift, from nanomaterials being considered completely novel and potentially requiring their own regulatory regime to the recent OECD Council Recommendation stating that nanomaterials can be safely regulated under existing regulatory frameworks, taking into account nano-specific considerations. To support existing regulatory programs, governments and jurisdictions are working together with their stakeholders, such as the scientific, industry, and NGO communities to ensure that decisions and approaches are scientifically robust. Examples of these activities will be highlighted in this presentation, which include: Canadian domestic activities such as the recent publicaation of the New Substances Program guidance on how new nanomaterials are considered under the New Substances Notifications Regulations (Chemicals and Polymers); The general agreement that categorization frameworks are appropriate for nanomaterials and the recent categorization work done under the Canada-US Regulatory Cooperation Council Nanotechnology Initiative and the OECD’s Working Party on Manufactured Nanomaterials on how and when to use categorization schemes; The activities of the OECD WPMN Steering Group on Risk Assessment and Regulatory Program (SG-AP) which has a mandate to develop risk assessment and risk management approaches to support the OECD Council Recommendation. These include applying existing approaches used for conventional chemicals to nanomaterials, developing new approaches, and looking at cross-cutting issues such as categories of nanomaterials to address risk assessment issues; and Transmissions from focusing on physical-chemical properties as predictors for environmental or population level effects to looking at behavioural tests together with physical-chemical properties. In addition, this presentation will draw parallels between the regulatory approaches used for conventional chemicals and nanomaterials and provide future directions on regulatory needs to conduct both screening-level and data-rich risk assessments of manufactured nanomaterials.

167 Adapting standard aquatic and sediment toxicity tests for use with manufactured nanomaterials: Key issues, expert consensus, and recommendations

S.A. Diamond, NanoSafe Inc. / Midwest Division; G. Goss, University of Alberta; K.T. Ho, U.S. EPA / Atlantic Ecology Division; J. R. Lead, University of South Carolina / Center for Environmental NanoScience and Risk; S.K. Hanna, National Institute of Standards and Technology / BioSystems and Biomaterials Division; K. Hunz-Rinke, Fraunhofer IME; A.J. Kennedy, CEERD-EPR; B.T. Mader, 3M / EHS Operations Environmental Laboratory; N. Manier, P. Pandand, INERIS; E. Petersen, National Institute of Standards & Technology; E. Salinas, BASF SE; P. Sayre, nanoRisk Analytics; K. Schwim, German Federal Environment Agency; D. Völker, Federal Environment Agency, Germany

The unique properties of manufactured nanomaterials (MN) suggest that their direct use or application in nano-enabled products will increase in the future. This will result in increased potential for human and environmental exposure to MNs during their manufacture, use, or disposal and suggests the need for MN-specific hazard and risk assessment approaches. Assessment of MN risk will require reliable, standardized hazard testing methods such as those provided by the Organization of Economic Cooperation and Development (OECD). However, guidance on how to design studies to address the many difficulties in testing MN, due to their particulate, fibrous, and colloidal properties, has yet to be developed. Here, we summarize findings from an expert workshop convened (as part of OECD’s Working Party on Manufactured Nanomaterials ongoing activities) for the purpose of developing such a guidance document for aquatic and sediment testing. Workshop attendees (23 experts from 6 countries) identified critical components of testing guidelines that will require additional or modified guidance. The discussion was based on attendees own research experience and on literature information elucidating the common issues related to material losses, dissolution, or modification in exposure systems and inconsistencies in testing practices. These include preparation of dispersions, exposure metrics, maintaining (and monitoring) consistent exposure levels, and reliable analytical and quantitative methods. In this presentation, we discuss these topics and identify where consensus was, and was not achieved including the heavily-debated issues of use dispersants,
manipulation of media properties (e.g. ionic strength or composition), testing unstable materials, using a water accommodated fraction approach, and adhering to the 20% exposure fluctuation thresholds (or applying other, MN-specific thresholds) common to many test guidelines. Workshop attendees also identified specific information gaps that might be addressed in future research to facilitate development of testing guidance. In addition to addressing standardized testing, the workshop findings will also facilitate more consistent testing and reporting of results in more exploratory research.

618 A transparent method to assess the regulatory adequacy of nanotoxicology studies

N. B. Hartmann, Technical University of Denmark (DTU) / DTU Environment; H. Lützhoft, Technical University of Denmark / Department of Environmental Engineering; M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry; A. Baun, DTU Environment / Department of Environmental Engineering

Environmental risk assessment of nanomaterials is required to ensure safety and protect the environment from unintentional adverse effects. In a regulatory context this requires reliable and relevant environmental hazard data upon which Predicted No-Effect Concentration (PNEC) values can be estimated. It is current practice that ecotoxicological studies are considered more valid for regulatory use if obtained according to accepted and validated test guidelines, preferably also following good laboratory practice (GLP). Evaluation of ecotoxicity studies is often done according to criteria specified by Klimisch et al. (1997), resulting in a so-called Klimisch score as a measure for study reliability. However, engineered nanomaterials (ENMs) are known to behave very differently in ecotoxicity test systems compared to soluble chemicals, for which most guidelines and the Klimisch method were intended. For this reason non-standard tests, or tests following modified test guidelines, may in some cases provide information of equal or higher reliability. Such data should therefore not per se be considered less reliable as basis for PNEC estimation. The aim of this paper was to develop a robust and transparent method to evaluate the reliability and relevance of ecotoxicity studies for nanomaterials. The development of this method draws on similar approaches developed for other groups of chemical and for evaluation of human toxicity data, as well as state-of-the-art knowledge on ecotoxicity test methods for nanomaterials. The result is a science-based method that is practical and flexible enough to accommodate different types of nanomaterials and aquatic ecotoxicity studies. The method is directly applicable in regulatory frameworks such as in risk assessment under the European chemical legislation, REACH. Klimisch, H. J., Andreae, M., & Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology : RTP, 25(1), 1-23. doi:10.1006/rtph.1996.1075

619 Ecotoxicity testing strategy towards development for grouping of nanomaterials

N. S. Luoma, University of California, Davis / John Muir Institute of the Environment; H. Lützhoft, Technical University of Denmark / Department of Environmental Engineering; M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry; S.N. Luoma, University of California, Davis / John Muir Institute of the Environment; H. Lützhoft, Technical University of Denmark / Department of Environmental Engineering; E. Salinas, BASF SE; M.T. Sørensen, ENVIRON International Corporation / Senior Science Advisor; J. Steevens, US Army Engineer Research and Development Center

Although engineered nanomaterials (NMs) have been the focus of intense research during the last couple of decades, several issues related to developing a scientifically based environmental risk assessment (ERA) framework for such materials within existing legislation still remain unsolved. Research so far suggests that there may be particle specific complexities demanding a more NM-targeted approach to estimating environmental exposures and testing toxicological effects. In addition, studies on effects from chronic and environmentally realistic exposures are scarce in the published literature. To address these issues an international workshop was held on November 9, 2014 in Vancouver prior to the SETAC NA meeting. Through seven invited talks and a panel discussion, two overarching charge questions were addressed: 1) Is it prudent to assess environmental risk based on current knowledge and understanding of NM fate and effects?, and 2) How may we direct future research to address potential uncertainties and data-gaps? In addition, participants acknowledged the pragmatic challenges in assessing NM risk based on existing methods and knowledge, and advocate that ERA is carried out with an understanding of identified uncertainties, challenges and limitations in NM-effect and exposure assessments. More specifically, since ERA should focus on what we are trying to protect, information may be needed beyond that found in published literature or produced by standard test guidelines, and therefore there is a need for development of alternative approaches to ERA. Efficient communication among researchers, regulators, and practitioners is essential to ensure effective assessments are conducted using appropriate endpoints and test conditions. This requires that the language and terminology used to describe fate, exposure, and biological effects of NMs, in addition to test procedures, mature to properly embrace specific particle complexities. In order to focus future NM research, it was generally agreed that one way forward could be to explore how models can be used to take a top down approach to identify: 1) what types of data and information are needed, 2) what parameters best predict nano-specific hazard and effects on protection goals, and 3) the information needed to make sense of the dynamic nature of different particle characteristics interacting

620 Challenges in the environmental hazard assessment of nanomaterials under REACH: Information in REACH registration dossiers for aquatic toxicity

L. Deyvier Stephan, European Chemicals Agency / Evaluation Directorate; A. Karki, European Chemicals Agency / ECHA Registration, Evaluation, Authorisation and Registration of Chemicals (REACH), even if having no special provision that refers to nanomaterials, covers nanomaterials of the substances. REACH Annexes from VII to X include standard information requirements for assessing environmental toxicity, fate and behaviour for all substances depending on the manufactured or imported quantities of the substance, including NMs. Dossiers registered under REACH regulation for substances known or detected as NMs were screened for acute and chronic aquatic toxicity results as part of the data available in the respective IUCLID dossiers. Around 20 dossiers and 15 different substances were analysed. Information in the aquatic toxicity studies including acute and long term toxicity testing on algae, aquatic invertebrates (mainly Daphnia) and Fish reported as key studies or supportive evidence were analysed. In addition, provided WOE (Weight of Evidence) to fulfill the information requirement or adaptations applied not to perform these tests were evaluated. The specific and general adaptations and information as key studies and test or preparations protocols have been analysed. Regardless of the data richness of the dossier, the reporting on the characteristics of the tested (nano)form, sample preparation, test performance and interpretation of the results was in many cases incomplete. In general, provided study summaries did not always thorough evaluation of the appropriateness of the tested nanoform or the quality of the performed test. Short term testing was preferred in the dossiers for testing aquatic toxicity and requirement for long-term testing often adapted referring to: lack of exposure, natural abundance, insolubility, or technical difficulties in testing. Based on the observations in the screening of information on the aquatic toxicity in the NM-related dossiers, a series of questions for adaptation and recommendations were made to improve the quality of the information for NMs. In conclusion majority of the challenges may be overcomed with proper documentacation of the sample characteristics, test protocols and interpretation of the obtained results with support of the foreseen adoption of new or updated test guidelines or guidance documents on protocols for dispersion and dissolution and ecotoxicity test protocols to be finalised within international organisation.

621 Output from a recent workshop on nanomaterial environmental risk assessment

N. S. Luoma, Roskilde University / Department of Environmental Social and Spatial Change; L.F. Baker, Baylor University / Department of Environmental Sciences; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; A. Gergs, RWTH Aachen University / Department of Environmental Social and Spatial Change; F. Von der Kammer, Vienna University / Department of Environmental Geosciences; S.N. Luoma, University of California, Davis / John Muir Institute of the Environment; H. Lützhoft, Technical University of Denmark / Department of Environmental Engineering; F. Salinas, BASF SE; M.T. Sørensen, ENVIRON International Corporation / Senior Science Advisor; J. Steevens, US Army Engineer Research and Development Center

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with environmental components. More detail on main workshop output will be presented.

Assessing the exposure: Prioritization, biomonitoring, modeling and experimental assessment of exposure factors (I)

622 Developmental neurotoxicity assessment of mixtures in children

P. Leondards, VU University, Institute for Environmental Studies / Institute for Environmental Studies IVM; M. Dingemans, IRAS Barcelona

Worldwide, serious concern has arisen about the increased incidence of learning and developmental disorders in children. From a scientific point of view, there is no doubt that exposure to neurotoxic chemicals during early brain development can adversely affect learning and development. Recent epidemiological and experimental studies have indicated that exposure to low doses of environmentally bioactive contaminants (e.g. pesticides) during human development can have deleterious effects on cognitive development in childhood. The European commission-funded project DENAMIC "Developmental Neurotoxicity Assessment of Mixtures in Children" investigates neurotoxic effects (e.g. learning and developmental disorders) of low-concentration mixtures of biologically active contaminants and a number of common environmental pollutants in children. We focus on (subclinical) effects on learning (cognitive skills) and developmental disorders in children (e.g. ADHD, autism spectrum disorders and anxiety disorders). One of the aims is to develop better and sophisticated tools, procedures and testing methods to screen compounds for (developmental) neurotoxicity and to improve assessment of exposures and effects (www.denamic-project.eu). As part of the project, a new alternative assessment strategy based on a combination of in vitro, in vivo assays and human exposure assessment is under development in order to prioritize compounds, and to further investigate the pathways and mechanism involved in disorders and diseases. The approach and results of DENAMIC will presented.

623 Multiscale connectivity - a high dimension biology approach to unravel the exposome

D.A. Sarigianis, S. Karakitsios, Aristotle University of Thessaloniki / Chemical Engineering

The exposome represents the totality of exposures from conception onwards, simultaneously identifying, characterizing and quantifying the exogenous and endogenous exposures and modifiable risk factors that predispose to and predict diseases throughout a person’s life span. Unravelling the exposome implies that both environmental exposures and genetic variation are reliably measured in tandem and linked through mechanistic analysis of toxicity pathways rather than only phenotypically associated. To better understand the interplay between environmental exposure and disease, we need to: (a) capture the biological perturbations initiated by exposure to environmental stressors; and (b) identify which of these perturbations overcome the homeostasis barrier, resulting in observed alterations of the cell/tissue environment and eventually to pathologic phenotypes. Towards this aim, integrated exposure biology provides the methodological elements for the surveillance of changes at different levels of biological organization through the use of the full array of –omics and post-omics technologies including epigenomics. Starting from untargeted transcriptomics and metabolomics we proceed with joint analysis of biological processes induced by exposure to xenobiotics at the molecular level and of metabolic processes induced in parallel. Dynamic flux balance analysis is a key element in the joint interpretation of gene expression data and metabolite profiles. This allows us to identify putative pathways of toxicity, which need to be verified by targeted –omics and functional assays. Identification of the functional links among the data derived from different high throughput testing platforms and their proper interpretation are supported by advanced bioinformatics such as support vector machines and clustering algorithms and systems biology models. Examples of the dawn of the exposure biology era are given and the future perspectives discussed in the context of supporting efficiently exposome studies.

624 Toxicogenomic analysis of ambient Particle Matter (PM) from rural and urban environments

S.R. Mesquita, Center of Marine and Environmental Research CIMAR / METOX Coastal and Marine Environmental Toxicology; B.L. van Drooge; E. Oliveira, IDAEA-CSIIC / Environmental Chemistry; J.O. Grimalt, Environmental Chemistry; C. Barata, CSIIC / Environmental Chemistry; N. Vieira, CIMAR and Faculty of Ciências da Saúde / University of Porto; L. Guimarães; CIMAR, University of Porto / METOX Coastal and Marine Environmental Toxicology; B. Pina, IDAEA-CSIIC / Environmental Chemistry

The toxic potential of the organic fraction of atmospheric particle matter (PM) of six sizes (>7.2; 7.2-3.1; 3.1-1.5; 1.5-1.0; <0.5 μm) was studied for an urban site in Barcelona and a rural site in the Pyrenees (NE Spain), in warm and cold periods between 2012 and 2013. A yeast-based assay for dioxin-like activity (AhR-RYA) was used to first screen the samples, and the zebrafish embryotoxicity (ZET) assay was used to analyze their in vivo toxicity. Dioxin-like activity was observed in both urban and rural samples, whereas only rural winter PM>0.5 μm samples induced severe deformations and mortality on zebrafish embryos, related to their very high content on organic compounds. Selected samples of urban and rural PM were used for the transcription analysis of exposed embryos, along with a positive control performed with the classic dioxin-like compound Benzo[a]Pyrene (BaP, 0.1ng/l). Transcriptomic analyses of zebrafish embryos exposed to BaP, urban and rural PM extracts showed a strong (and similar) induction of the AhR, a high dimension biology approach to unravel the exposome.
worldwide. Concerning PBDEs, their concentrations rank amongst the highest in the world, with the exception of North America and highly-polluted sites. Age, sex and the body mass index are strong determinants in the patterns of accumulation of OCs and PBDEs. The concentrations of OCs increase with age while the concentrations of PBDEs are higher in young individuals (Sexual differences are also observed: with increasing age, women have higher concentrations of several OCs, particularly HCB and \( \beta \)-HCH. Furthermore, the influence of the body mass index in the accumulation of these compounds is proportionately higher among men than in women. These differential trends may probably lie in the conjunction of both the physical-chemical properties of these compounds and the dynamics of human metabolism. Other socio-demographic determinants such as educational level, social class and place of birth contribute less to explain the accumulation of POPs in the studied population.

**Effect Modelling of environmental systems - extrapolation and prediction of adverse outcome (I)**

627 Assessing data needs, precision of parameter estimates and goodness-of-fit of model predictions for TK/TD models: chlorpyrifos as a test case

A. Focks, Alterra Wageningen University and Research Centre / Ecotoxicology Environmental Risk Assessment Team; M.N. Rubach, Syngenta Crop Protection AG / Global Regulatory; M. Zafar, Quaid-i-Azam University; R. Ashauer, University of York / Environment; P.J. van den Brink, Alterra and Wageningen University.

Toxicokinetic-toxicodynamic models provide exciting opportunities in linking exposure with effects. These opportunities are accessible, however, only when the models are properly parameterized, an exercise still necessary for each and every combination of species and chemical. The data needs and related effort for parameterising TK/TD models were considered to be quite high for long times. Recently, Nyman et al. investigated the influence of the availability of internal concentration data on the goodness-of-fit of survival probabilities for propiconazole in *Gammarus pulex* for different TK/TD model types. They found that the goodness-of-fit of model predictions did not improve significantly if data on internal concentrations were considered for the parameterisation. Based on a comprehensive sensitivity analysis, they concluded Chlorophyll a and propiconazole extended the questions of Nyman et al. by additionally considering whether absolute values and confidence intervals of estimated model parameters are dependent on knowledge about toxicokinetics. The dataset offered the unique opportunity to analyse related questions for a set of 14 macroinvertebrate species and to test the goodness-of-fit of different model parameterisations by using survival data for 6 of those 14 species under pulsed exposures. More information is given in the extended abstract.

628 Can toxicokinetic and dynamic modelling be used to understand, quantify and predict synergistic interactions between chemicals?

N. Cederghen, K. Uffmann, M. Gottardi, University of Copenhagen / Department of Plant and Environmental Sciences; A.C. Kretschmann, University of Copenhagen

Mixtures of toxic chemicals may exhibit synergistic or antagonistic effects. Understanding the mechanisms and magnitudes of synergistic responses of aquatic organisms to mixtures of toxic chemicals is a significant challenge for ecotoxicology. This is particularly true for complex mixtures of contaminants from synthetic organic chemicals (SOCs) and POPs as well as from natural organic matter (NOM), which is also a major source of aquatic toxicity. The aim of this study was to understand how the time course of effects is influenced by the presence of mixtures of toxicants.

629 How toxicokinetic and toxicodynamic processes contribute to the time dependency of toxicity? - A modeling case study for different adverse outcome pathways

C. Vogt, Department Bioanalytical Ecotoxicology, R. Altenburger, UFC Centre for Environmental Research / Department of Bioanalytical Ecotoxicology

Abstract The time course of toxicological effects of chemicals may result from toxicokinetic and toxicodynamic processes. This study seeks to characterize whether toxicokinetics or toxicodynamics dominates the perturbed growth of the green algae *Scenedesmus obliquus* over different kinetic time scales and adverse outcome pathways. To this end, comprehensive data of internal effect concentrations and the related affected growth profiles were used for effect modeling of concentration-time-response relationships. The choice of six model chemicals was based on the expected time to reach equilibrium concentrations in the algae cells according to a hydrophobicity-driven partition process combined with the suspected time to progress the effect from the initiating event to the adverse outcome through key toxicity pathways. As a result, delayed toxicity occurred to be mainly dominated by the toxicodynamic processes. The predicted kinetic rates characterizing the effect progression ranged over six orders of magnitude in the following order: inhibitor of the photosystem II > reactive chemicals > inhibitor of lipid synthesis. Kinetics of effect progression were quantified to be specific for different adverse outcome pathways and seemed to be independent of whether equilibrium concentrations in the algae were reached over minutes or hours. The determination of effect progression rates might help to characterize and distinguish different adverse outcome pathways. Keywords: phytoxicity, modes of toxic action, toxicokinetic-toxicodynamic modeling, hypothesis-based experimental set up.

630 Death dilemma and organism recovery: toxicodynamic patterns in chemical space

R. Ashauer, University of York / Environment; I.A. O'Connor, Pfizer Pharmaceuticals; A. Hintermeister, TESTEX AG / Analytics Department; B.I. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology

Why do some individuals survive after exposure to chemicals while others die? Either the tolerance threshold is distributed amongst the individuals in a population, and its exceedance leads to certain death, or all individuals share the same threshold above which death occurs stochastically. Both assumptions lead to the typically observed sigmoidal dose-response curves, but the time course of mortality differs markedly. More importantly, if one were to assume stochastic death one would conclude systematically stronger compensation and damage repair mechanisms than with the assumption of individual tolerance. Thus we face a circular dilemma because inference about the death mechanism is inherently linked to the definition of tolerance. Therefore, we aimed to establish a mathematical relationship between the two assumptions and resulted in the General Unified Threshold model of Survival (GUTS). In previous work we experimentally quantified the uptake, biotransformation and elimination rates of 14 synthetic organic chemicals in the aquatic invertebrate Gammarus pulex and built a toxicokinetic model. But now we were able to construct a toxicokinetic-toxicodynamic model for each compound where the toxicokinetic part simulates the time course of the internal concentrations. The sums of internal concentrations of the toxicologically active chemicals were the inputs for the toxicodynamic model part. We include mixture effects by explicitly modelling the biotransformation products and their contribution to toxicity. Stochastic death (SD) and individual tolerance (IT) were about equally likely. By separating toxicokinetics from toxicodynamics we studied the position of chemicals in the toxicodynamic parameter space and found patterns and clusters amongst chemicals related to modes of toxic action. Thus we suggest toxicodynamic parameters as novel phenotypic anchors for in *vitro* to in *vivo* toxicity extrapolation. For all chemicals IT was associated with smaller recovery rates than SD, which confirms the expectation that organism recovery is slower in case of IT as compared to SD. This means that any given survival data can be explained by either slower organism recovery and a wider individual tolerance distribution, or faster organism recovery paired with a narrow tolerance distribution in the test population. What’s more: GUTS debunks the myth of asymptotic LC50s.

631 Species sensitivity and metabolic rate

J. Baas, Centre for Ecology & Hydrology; S.A. Kooijman, Vrije Universiteit

Ecotoxicological studies have shown considerable variation in species sensitivity for chemical compounds, but general patterns in sensitivity are still not known. A better understanding of this sensitivity is important in the context of environmental impact assessment but also in a more general ecology and evolutionary one. We investigated the metabolic rate or more precisely the specific somatic maintenance (expressed in J.cm\(^{-2}\cdot\)d\(^{-1}\), at a standardised body temperature of 20 °C) on the sensitivity of a species to chemical poisoning. The sensitivity of a species was expressed in terms of its threshold concentration for survival in the simplest toxicokinetic-toxicodynamic model available, the No Effect Concentration (NEC, expressed in \(\mu\)g/L). Somatic maintenance data were based on the ‘add-a-pot’ database hosted by the VU University of Amsterdam. NECs were derived from the US-EPA ECOTOX database. We focussed on 4 pesticides; 2 that need a metabolic activation, Chlorpyrifos and Malathion, and two without metabolic activation, Carbofuran and Carbaryl. All 4 pesticides showed a similar response in over 50
different species in total: a strong negative correlation between the specific somatic maintenance and the NEC. This intuitively makes sense, species with a higher metabolic rate are more susceptible for interference with toxicants. The metabolic rate is not the only trait that makes species more or less sensitive to toxicants, however using species traits to get a first appraisal on their sensitivity to chemical stress is a promising line of research

632 Inter-species and inter-level transferability of ecotoxicological test results A. Gerbs, RWTH Aachen University / Department of Environmental Social and Spatial Change; T.G. Preuss, Bayer CropScience / Environmental Modelling Environmental risk assessment of chemicals is largely based on adverse effects measured in organisms indicating to standart conditions. Protection goals for environmental risk assessment usually include non-target species as well as the populations and communities they constitute. Interspecies differences and interactions between chemical, physical and biological factors are therefore generally unaccounted for. Uncertainties in the risk assessment procedure are thus related to the extrapolation of ecotoxicological test results to non-standard species and higher level protection goal such as populations. To bridge this gap between effect measures and protection goals and to account for various species in variable environments, we need predictive modeling approaches. Therefore, process-based mechanistic effect models, that take underlying physiological and ecological mechanisms into account, are gaining resonance in scientific and regulatory communities as a means to bring more ecological realism and more integrated predictions into ecological risk assessment of chemicals. In this regard, we raise the question if effects on populations that belong to a certain species are predictable from effect measures derived from ecotoxicological tests using a different species. In a toxicokinetic-toxicodynamic modelling approach we assume that differences in effects among species and among different life stages within a species are the result of differential toxicokinetics, i.e. the process translating exposure to body burdens. We tested this hypothesis on acute tests employing three aquatic species, the water flea Daphnia magna, the copepod Mesocyclops leuckarti and the larval phantom midge Chaoborus crystallinus, exposed to triphenyltin. In a second step we extrapolate acute effects observed for M. leuckarti and C. crystallinus to populations of D. magna by integrating the toxicokinetic-toxicodynamic model in an individual-based population model framework. The approach is evaluated by confronting model predictions with independent population data. While we conclude that the inter-species- and inter-level extrapolation of ecotoxicological test results is feasible to some extent, we also discuss limitations for the presented model.

Addressing data collection and computational challenges in LCI (l)

633 TOWARDS A GLOBAL NETWORK OF INTEROPERABLE LCA DATABASES L. Mila i Canals, UNEP; A. Asselin, UNEP DTIE Paris / SCP; H. Cruypenninck, UNEP-DTIE; S. Liu, USDA; W. Ingwersen, US EPA; T. MacDonald, U.S. Environmental Protection Agency / Office of International Affairs Introduction. Meetings on international LCA cooperation have been held since 2012 among governmental and intergovernmental organizations towards advancing the development and application of life cycle assessment as a tool for sustainability in decision making and public policy. Within this framework, a meeting organized by UNEP, US EPA and USDA was held in April 2014 in Washington D.C., specifically focused on LCA data and databases with more than 60 participants (governments and LCA database experts) from 17 countries. Purpose. The objective of the meeting was to develop a vision, guiding principles and an approach for implementing a voluntary international network to promote LCA data accessibility, interoperability and applications, and define a proposed work plan for moving such an initiative forward. Results. The two-day interactive workshop on LCA data and database interoperability, and follow-up governmental meeting to consider proposals from the workshop (as well as review experience of LCA applications in public policy, other data quality issues, and capacity building initiatives) resulted in an executive summary including proposed “Next Steps on Building a Global Network of Interoperable LCA Databases.” Key elements included a proposed governance structure to help create a global LCA database network, as well as a proposed set of early deliverables. Results related to governance included: Forming an interim planning group (IPG) composed of governments and international organizations to better defining the initiative and process for a long term LCA network steering committee, and its 10 working initiatives, while adding innovative elements for raw data harvesting, data consolidation, validation, review, system modelling, uncertainty propagation, and user interfaces. The current challenges and status of the initiative and its 10 working groups are presented.

635 Towards a global regionalised inventory of thermoelectric power plants and their emissions C. F. Raptis, Institute of Environmental Engineering; S. Pister, ETH Zurich The electricity industry has been the focus of many LCA studies, owing to the many different impacts associated with their operation. Nowadays it is difficult to imagine a product or process that does not involve electricity production, therefore, being able to access a global, regionalised inventory of emissions per unit of energy produced becomes all the more important. In this work we present the first steps and results of our holistic and systematic approach to creating a global, spatially explicit inventory of the thermoelectric power plants, based on analysis of the data available in the Platts World Electric Power Plant (WEEP) database. Starting by systematically georeferencing the power plants we proceed to carry out a computationally intensive data analysis, permitting the calculation of the thermal efficiency of each individual thermoelectric generating unit. These thermal efficiencies are improved compared to generic estimates based on the fuel used, often encountered for thermoelectric plants and can be further employed in the calculation of emissions important to LCA, or ones never calculated so far, such as freshwater thermal emissions. We present the steps in the data analysis, followed by the results for both thermal efficiencies and freshwater thermal emissions per power plant technology, fuel type and MJ of electricity produced on a 0.5x0.5 degree grid scale with global coverage. Freshwater thermal impacts are expected to be highly regional, making an analysis on this level of geographical detail crucial. Preliminary results for a fraction of thermoelectric power plants using once-through cooling systems and subcritical steam conditions show that the highest absolute freshwater thermal emissions come from nuclear power plants, but that the fraction of heat rejected/electricity produced is similar between the power plants of this category. As an example for coal-fired power plants on average 2.4 MW are emitted into freshwater for every MW of electricity produced, whereas for nuclear power plants this value is equal to 2.9 MW/MW. We analyse the variability in all results in terms of fuel and technology type, and in terms of the location of the power plant. We discuss the advantages and limitations of our approach, with particular focus on the need to fill in patchy data, and we present our future steps in completing the global, regionalised set of emission data for thermoelectric power plants.
implementation of an information retrieval method used in popular web search engines (query expansion with background knowledge). We applied our method to produce crop life cycle inventories for the whole of Australia at 1km2 resolution, and validated modelled fertiliser, pesticide and fuel use against the best available datasets at the national and local scales. The model covers the 72 highest ranked broad-acre and horticultural crops in terms of area coverage and economic value in Australia. Its results can be scaled up to assess the baseline environmental impacts at the landscape, regional or national level. Our method delivers valuable information for on-farm decision making and contributes to the design of more cost-effective policies around sustainable intensification or land sparing by providing policy-makers with a greater understanding of the relationship between small-scale actions and large-scale impacts.

637 A Novel Approach for Inclusion of LUC from Biofuels in Consequential LCA

Y. Cikovani, Technische Universiteit Darmstadt / Civil and Environmental Engineering; L. Schebek, Technische Universiteit Darmstadt / Material Flow Management and Resource Economy; R. Schaldach, F. Wimmer, University of Kassel CESR

Keywords: biofuels, spatially explicit land use modelling, computational methods

Biofuels are considered a major option for the mitigation of climate change and have therefore been included in climate policies worldwide. However, several studies have revealed that the benefits for agricultural biomass as feedstock for biofuels may induce land use change (LUC) and might even offset the savings of greenhouse gas (GHG) emissions by the replacement of fossil fuels. Studies based on these approaches give valuable insights, but generally on a spatial very aggregated level. To overcome these limitations and to include sophisticated data in LCI within CLCA, we developed a novel approach of coupling LCA with the spatial explicit land use change simulation model LandSHIFT (Land Simulation to Harmonize and Integrate Freshwater Availability and the Terrestrial Ecosystems) with an automated linking approach to incorporate interfaces between all tasks of concern. The model input is a set of exogenous drivers such as population and production of agricultural commodities. The model output is a time series of high resolution raster maps of the changing land use pattern. The LandSHIFT output data, i.e. output raster maps, can be processed within LCA for a determined scenario. Benefit of this approach is that GHG emissions not only can be investigated as to different demand scenarios, but also within one and the same scenario, impacts and effects of relevant parameters - such as the influence of technology efficiencies, changes in land productivities, influence of feedstock and technology compositions etc. - can be evaluated in order to provide specific on-site recommendations for action. Summing up, this computational approach enables the framework of CLCA factoring in socioeconomic as well as biophysical aspects. The approach integrates a computational tool outside of the LCA space, yet is coupled to LCA and can improve sophistication of LCI data. Thus, the generated data is implemented as to a parameterization of conventional LCA-database. This approach therefore supports decision making on a regional level and facilitates governance of exogenous drivers by analysing management regimes to regional stakeholders and brings forward the current insights regarding spatial, temporal and uncertainty issues in a holistic manner.

638 Life Cycle inventory of chemical emissions during product use: A parsimonious emission model of chemicals encapsulated in products

L. Huang, University of Michigan / Dept of Environmental Health Sciences School of Public Health; O. Jollivet, University of Michigan / Environmental Health Sciences School of Public Health

Current approaches for life cycle inventory calculations in life cycle assessment (LCA) have primarily focused on the impacts of outdoor emissions or the manufacturing stage without considering near-field releases and exposures during product use. However, studies have demonstrated that near-field chemical intakes may exceed environmentally mediated exposures and are therefore essential to be considered when assessing chemical emissions across a product’s life cycle. Chemicals encapsulated in materials/products can be a major emission source in the use-phase. This is for example volatile organic compounds (VOCs) in building materials and flame retardants in furniture/consumer products. Previous models describing such emissions require complex analytical or numerical solutions, which poses a great computational burden and lack transparency. In the present study, we adapted a model which describes VOC emissions from building materials and subsequent loss by ventilation, and simplified the governing equations by accessing steady-state emissions and the solution of the simplified governing equations for total mass emitted, which consist of a sum of infinite exponential terms (2000 – 5000 terms are needed to be accurate), was further reduced to a sum of only two exponentials. Results show that this simple two-exponential solution can approximate the real total mass emitted over 15 years with R-square greater than 0.99 for a wide range of compounds. This approach greatly reduces the computational burden, and can be easily implemented in Excel, which is suitable for high-throughput modeling. Based on this approach, the emissions of chemicals encapsulated in products can be reported in the inventory either as the quantity of chemical inside a product over a certain time, with subsequent determination of product intake fraction in LCIA, or as indoor releases, followed by application of the USETox indoor model.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (I) 639

Which Aquatic Invertebrate Tests should be in the OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors? R.S. Bensted, Food and Environment Research Agency / Centre for Chemical Safety and Stewardship

The OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors (2012) sets out a framework for assessing the risk of chemicals having activities able to interact with the Endocrine, Anti-androgenic, Thyroidal and Steroidal (EATS) pathways in vertebrates. It proposes that two aspects are determined before a chemical can be referred to as endocrine disruptive. Firstly, the mode of action must be demonstrated to interfere with these pathways (by in vitro testing, or in vivo using biomarkers etc.), and secondly such effects should have a measurably adverse outcome. Invertebrates are also included in the Framework, but as yet, no tests are available for assessing EATS modes of action in these groups. In the absence of this information, this project makes an assessment of the responses of species and clades of invertebrates to compounds known to control these pathways in vertebrates, in order to identify those species that are most suited to use in the Conceptual Framework for the protection of invertebrate populations from chemicals regarded as endocrine disruptive under this definition. Research into relevant modes of action in invertebrates is in its infancy, but this should not defer the assessment of such chemicals in these organisms, and where there is a clear need to include invertebrate tests in the Conceptual Framework, these should be developed and proposed for regulatory risk assessment. Conversely, where invertebrate tests are not relevant for the purposes of the Conceptual Framework, these should not be included. In addition, the Guidance Document that includes the Conceptual Framework (OECD Guidance Document 150) states that ‘the interpretation of results from invertebrate test guidelines is not included due to the rather poor current understanding of endocrinology in most invertebrates, and the lack of diagnostic screening endpoints with these taxonomic groups.’ If the recommendations from this project are adopted, then interpretation of the invertebrate tests will be easier, since responses will be aligned to recommended wide-scale responsiveness in the invertebrate group used in testing, and the apical end-points measured will be conceptually linked to those observed in vertebrates.

640 Targeted analytical toxicology: Simultaneous determination of 17α-ethynylestradiol and the estrogen-induced vitellogenin biomarker F. Yang, W. Huang, W. Xie, Zhejiang University; C. Lu, Harvard School of Public Health / Environmental Health, W. Liu, Zhejiang University / College of Environmental and Resource Sciences

In most toxicological studies, chemical concentrations and its biological/molecular response (biomarker) levels are often quantified separately. We report a method, named as Targeted Analytical Toxicology (TAT), for simultaneous determination of levels of chemicals and protein biomarkers in organisms by UPLC/ESI-MS/MS on the basis of targeted proteomics. The TAT method is successfully applied to detect the concentrations of 17α-ethynylestradiol (EE3) and EE2-induced vitellogenin (a biomarker for estrogens) in male zebrafish exposed to EE2. It is expected for TAT to have multiple applications in toxicology, especially environmental toxicology, due to its high sensitivity, accuracy, reproducibility, high throughput, and the savings of time, cost and labor.

641 A frog is a frog...or is it? Variation in response of Xenopus laevis to oestrogen T. Hayes, UC Berkeley / Integrative Biology

Despite the acceptance of the African clawed frog (Xenopus laevis) as a laboratory model to examine endocrine disruptors, little is known about intraspecific variation in the response to hormones and endocrine disruptors in this model. In the current study, we evaluated the response of X. laevis to estradiol 17β (E2) using multiple sources of X. laevis. We identified significant variation in the response to E2 between populations of X. laevis. Animals from a commercial source (Nasco) and from a feral population from San Diego were very sensitive to E2 and responded to E2 concentrations as low as 30 pg/ml with genetic males developing ovaries. Animals from other commercial sources (Xenopus Express and Xenopus One) were least sensitive to E2 and did not respond until a concentration 100 times higher (3 ng/ml). At 3 ng/ml differences in sensitivity were also observed. In animals from a feral population from San Diego, up to 90% of the exposed males developed ovaries, compared to less than 15% sex reversal in all other populations, except the San Francisco population where E2 exposure at this concentration resulted in 50% hermaphrodites. Examination of multiple clutches per pair demonstrated that results were repeatable across clutches. Repeating experiments at 17 and 27 °C showed that temperature did not impact the effect of E2 on sex differentiation. Examination of multiple pairs within each population showed that the differences in sensitivity were repeatable within a population, with the exception of the poulation from Potchefstroom, where larvae from different pairs showed a range of sensitivity.
that overlapped with populations deemed sensitive and insensitive. These data demonstrate that there is significant variation in sensitivity to estrogen between sources of *X. laevis*. Given the popularity of *X. laevis* as a model for examining endocrine disruptors, these findings are significant.

### 642 Are all chemicals endocrine disruptors?

J.R. Wheeler, Dow AgroSciences; S. Marty, Dow Chemical Company; E.M. Mihaich, ER2; L.S. Ortego, Bayer CropScience / Environmental Toxicology and Risk Assessment; K.K. Caudy, The Dow Chemical Company / Toxicology Environmental Research and Consulting; L. Zorrilla, Bayer CropScience LP

Endocrine disrupting (ED) properties require evaluation under the European REACH regulation, as well as for regulation of plant protection and biocidal products. Specific criteria is under development in the EU to identify ED properties to enable hazard-based regulation, while in the US and Japan, risk-based approaches are being developed. Irrespective of the regulatory process, most geographies use the WHO IPCS definition, or variants thereof, requiring that a substance is demonstrated to cause a change in endocrine function that consequently leads to an adverse effect in an intact organism to identify it as an endocrine disruptor. Such a definition is broad and at its most cautious might capture many mechanisms that in general would not specifically be considered ED. For instance, stress is a non-specific, neuro-endocrine response that can lead to adverse outcomes. In addition, other toxic mechanisms (e.g. liver toxicity) may also secondarily impact the endocrine system and tissues. Furthermore, endocrine responses may be adaptive in nature, designed to maintain homeostasis, rather than inducing an irreversible adverse effect. Such factors should be considered when screening and testing substances for potential endocrine activity or disruption.

Following the large scale screening of pesticides and pesticide inerts under the USEPA's Endocrine Disruptor Screening Program, practical experience with screening assays has highlighted some of these factors as important to data interpretation and study design. Clearly, the misidentification of indirect effects as truly ED has serious consequences in terms of triggering unnecessary higher testing (animal and resource intensive) and potentially severe regulatory consequences in the EU (removal from the market). A review of effects that could be misinterpreted as ED was undertaken using both practical experience with the assays and relevant, peer-reviewed, scientific literature. Focus was on the endocrine mechanisms of action (fish short reproduction and amphibian metamorphosis) while drawing parallels to mammalian toxicology. This work demonstrates that without a suitable framework for interpreting study results, potentially all chemicals could be considered ED at some exposure level, if too broad a definition of endocrine disruption is used. This presentation highlights the problem of distinguishing non-endocrine from endocrine mechanisms of action when operating in a purely hazard-based regulatory environment.

### 643 Hazard versus Risk Assessment of Endocrine Disruptors: A Communications Challenge

T.A. Verslycke, L. Zorrilla, T.A. Verslycke, Dow Chemical Company; L. Zorrilla, Dow Chemical Company

While hazard and risk are terms commonly used in everyday life, they are often misunderstood by the general public and poorly communicated by academia, industry, government, non-profits, and the media. There is certainly no lack of examples where public perception and outrage over environmental health risks has been at odds with the scientific assessment of these same risks. Similarly, chemical hazards and chemical risk are two distinct terms that are generally well understood by environmental toxicologists and chemists (i.e., the “SETAC community”), but are difficult to communicate beyond (and sometimes even within) this community.

In an age of growing consumer outrage over environmental health risks, it is increasingly challenging to effectively communicate hazards and risks, as well as potential solutions to mitigate them. For instance, stress is a non-endocrine response that can lead to adverse outcomes. In addition, other toxic mechanisms (e.g. liver toxicity) may also secondarily impact the endocrine system and tissues. Furthermore, endocrine responses may be adaptive in nature, designed to maintain homeostasis, rather than inducing an irreversible adverse effect. Such factors should be considered when screening and testing substances for potential endocrine activity or disruption.

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### 644 Are non-monotonic dose-response curves relevant to regulatory decision-making?

J.P. Myers, Environmental Health Sciences; L. Vandenberg, Tufts University Endocrinology has acknowledged the existence of non-monotonic dose response curves as a basic attribute of hormone signaling for decades. Many examples have now been published in peer-reviewed studies of endocrine-disrupting compounds. This phenomenon, however, has yet to be incorporated in regulatory toxicology in the determination of chemical safety standards. We conducted thought experiments on whether non-monotonicity could interfere with calculation of determination of safe levels of exposure using standard procedures of regulatory toxicology considering three cases: (1) non-monotonicity occurs at high levels of exposure; (2) non-monotonicity takes place around the level that has been determined as the no-observed adverse effect level (NOAEL); and (3) non-monotonicity occurs within the range of the reference dose established by high dose experiments. We also reviewed the peer-reviewed literature to determine whether these conditions of each case actually occur. We found multiple examples of each case. We conclude that non-monotonicity is problematic for calculating safe levels of exposure because it occurs regularly at concentrations that would prevent its detection by regulatory toxicology as it is commonly practiced today.

#### Ecotoxicology in tropical and polar regions (II)

### 645 Biological indicators for the impact assessment of (new) human activities in the Arctic


Marine sediments often act as a sink for bioaccumulative persistent chemicals, including oil or petrogenic PAHs. Sediment PAH composition may be altered due to varying resulting in a higher PAH mixture at each location. We found multiple examples of each case. We conclude that non-monotonicity is problematic for calculating safe levels of exposure because it occurs regularly at concentrations that would prevent its detection by regulatory toxicology as it is commonly practiced today.
biss-2,ethyl-1-hexyltetramorphothalate (BETHBP) and biss-2,ethyl-1-hexyltetramorphothalate (TBPH), were frequently detectable in air at the Canadian Arctic station of Alert (82°30’N, 62°20’W). At the Yukon station of Little Fox Lake (61°21’N, 135°38’W), DT, decachlorobenzenes 602, pentabromotoluene (PBT), 2,3-dibromopropyl-2,4,6-trichlorophenyl ether (DAPTE), and hexabromobenzene (HBB) were frequently detected. High concentrations of OPFRs have been observed in the Antarctic environment, as well as the larger PCB compounds found in the Antarctic environment, and as vectors for the long range environmental transport of pollutants. Levels of OPFRs, including tri-chloroethyl phosphate (TCP), tri-chloroethyl phosphate (TCEP), tri-chloroethyl phosphate (TCEP), triphenyl phosphate (TPh), ethyl-diphenyl phosphate (EHDP), and tris-dichloropropyl phosphate (TDCPP), measured on cruises were higher than all other FRs in the Arctic. OPFRs arrive in the arctic sorbed to small particles.

464 Immunodulatory effects of In vitro and In vivo exposure to PCBs and perfluorooctane sulfonic compounds in East Greenland ringed seals M.J. Levin, University of Connecticut; J.W. Desforges, Aarhus University (AU) / bioscience; E. Gabbard, University of Connecticut; R. Dietz, C. Sonne, Aarhus University / Department of Arctic Environment; R. Bossi, Environmental Science; R.J. Letcher, Environment Canada / Ecotoxicology and Wildlife Health Division; K. Gabrielsen, Norwegian University of Science and Technology / Biology; M.M. Jønssen, Norwegian University of Science and Technology / Dept of Biology; S. De Guise, University of Connecticut / Department of Pathobiology and Veterinary Science

To better elucidate the potential immune-related health effects of contaminant exposure on the health of sentinel arctic species, we collected high-quality tissue samples from 14 ringed seals (Pusa hispida) during the 2012 aboriginal subsistence harvest in East Greenland. We measured the concentration-response effects of in vitro exposure to various PCB congeners and perfluoralkylated substances on mitogen-induced lymphocyte proliferation. Neither perfluorooctanoic acid (PFOA) nor perfluorooctanesulfonic acid (PFOS) modulated lymphocyte proliferation in the exposure range of 0 to 300 ppb. The non-co-planar PCB congeners CB-138, -153, and -180, but not the coplanar CB-169, reduced ringed seal lymphocyte proliferation between 10 and 20 ppm using in vitro exposure. Serum levels of PFOA correlated with the concentration of cytokines measured from the supernatant of cultured lymphocytes; IL-12 (r=0.63, p<0.05) and IL-18 (r=0.83, p<0.05). Serum levels of PFOS and PFOA in free-ranging seals did not correlate with the proliferative response of lymphocytes. Concentrations of PCBs found to modulate immune function in this study are above the range measured in free-ranging ringed seals but within the range of other arctic marine mammals such as polar bears (Ursus maritimus) and killer whales (Orcinus Orca), suggesting top predators are at risk of exposure to environmental exposure of contaminants if they are as sensitive as the studied ringed seals. These results add to our understanding of the impacts of global pollution on arctic top predators and highlight the risk of increased susceptibility to novel pathogens and reduced overall health due to immunosuppression.

468 The role of bird species in biological focussing of Persistent Organic Pollutants and as vectors for the long-range environmental transport of pollutants to the Antarctic S.J. Wild, Griffith University; I. Eulaers, University of Antwerp / Biology; A. Covaci, University of Antwerp, Toxicological Centre / Toxicological Centre; H. Van der Veken, Griffith University / School of Environment; R. Crupp, Griffith University; J. Dachs, IDAEA-CSIC / Toxicological Centre; J. Pastores, Griffith University / School of Environment; M. Bigot, University of Antwerp, Toxicological Center / Toxicological Centre; D. Hawker, Griffith University / School of Environment; R. Cropp, Griffith University; J. Dachs, IDAEA-CSIC / Environment Chemistry; C. Teixeira, Environment Canada; S.M. Bengtson Nash, Griffith University / School of Environment; R. Cropp, Griffith University / School of Environment; R. Bossi, Environmental Science; R.J. Letcher, Environment Canada / Ecotoxicology and Wildlife Health Division; K. Gabrielsen, Norwegian University of Science and Technology / Biology; M.M. Jønssen, Norwegian University of Science and Technology / Dept of Biology; S. De Guise, University of Connecticut / Department of Pathobiology and Veterinary Science

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649 Organochlorine pesticides in the marine environment of the Southern Ocean (Indian/Pacific Ocean sector) M. Oeggerli, Griffith School of Environment; D.C. Muir, Environment Canada / Aquatic Contaminants Research Division; D. Hawker, Griffith University / School of Environment; R. Cropp, Griffith University; J. Dachs, IDAEA-CSIC / Environmental Chemistry; C. Teixeira, Environment Canada; S.M. Bengtson Nash, Griffith University / Southern Ocean Persistent Organic Pollutants Program SOOPPP

Persistent Organic Pollutants (POPs) are ubiquitous anthropogenic chemical substances that have been widely used in agricultural and manufacturing industries. These compounds, which include a variety of organochlorine pesticides (OCPs), are highly resistant to biodegradation, capable of long-range environmental transport, are toxic and bioaccumulate in living organisms. The Stockholm Convention is a legally binding international treaty that seeks to ban or severely restrict the use and release of POPs into the environment to minimise the hazard they pose. Not all nations manufacturing POPs, however, are signatories. Many POPs are semi-volatile in nature. This has led to primarily atmospheric transport of part of the global POP burden to remote Polar Regions. At these cold conditions at high latitudes, their diminished volatility serves to restrict further movement. Global warming, however, stands to significantly alter the Polar climate and may cause POPs to be released from the polar icecaps into marine and terrestrial regions where there is currently net air to water flux. So-called “polar sinks” may, under this scenario, become secondary sources of POPs to the atmosphere, thus impacting global efforts to moderate human and environmental exposure to these toxic compounds. POP research in the Southern Ocean context remains insufficient to gauge the current magnitude of the Southern Ocean POP reservoir and further, to estimate the current net directional movement of chemicals at the air / ocean interface. Whilst a number of recent studies have focused on the POP burden of the marine environment of the south Atlantic sector of the Southern Ocean, limited studies have been conducted in the south Pacific sector in the past two decades. Here we present new knowledge regarding current levels and profiles of OCPs in the marine environment of the Indian/Pacific Ocean sector of the Southern Ocean. The study facilitates an improved understanding of OCP distribution in this global region. Environmental samples were collected along a transect from Australia to Antarctica on board the RSV Aurora Australis in February 2014. They were analysed for a suite of 31 organochlorine pesticides. Results of this sampling campaign will be presented and discussed.

Challenges and Opportunities in Protecting Human and Ecosystem Health: Understanding the Fate, Transport, and Toxicity of Wastewater-borne Contaminants (II) S. Manzoor, A. Korre, Imperial College London / South Kensington Campus / Earth Science and Engineering; A. Simperler, Imperial College London / South Kensington Campus / Department of Chemistry

Amine solvents used for post combustion CO2 capture (PCC) produce compounds in the atmosphere, such as nitrosamines (NS) and nitramines (NA) that are detrimental to human health. The interaction of POPs, including OPFRs, organochlorine pesticides (OCPs) and brominated flame retardants (BFRs) and OCPs with these compounds changes the atmospheric chemistry with dispersion calculations to assist in performing human health risk assessments remains a key knowledge gap. In this study, rate coefficients established through quantum chemical theoretical methods and kinetic modelling describing complex atmospheric chemistry mechanisms of the most commonly used amine for CO2 capture, Monoethanolamine (MEA) and the degradation products, namely methylamine (MA) and dimethylamine (DMA), are considered with dispersion studies for the UK’s largest pilot plant, CCPilot 100+ in Ferrybridge, in the atmosphere using an air-dispersion model, ADMS 5. The developed methodology enables in determining reliable concentration of the resulting NS and NA in the atmosphere and their transport, dispersion and deposition away from the plant in time and space. Applying it to a worst case scenario of reactive amine emissions from a PCC plant assistance assessing the risk posed to the human health by comparing the reported as well as calculated safety limits. It is therefore suggested that this approach is reliable in determining the risk these amine emissions from PCC technology pose to the humans. This methodology is applicable to any size of power plant and at any geographical location
Ecological fate and assessment of home and personal care products in Asia: Ecohope
C. Wannaz, The University of Michigan, Ann Arbor / SPHEHS; A. Franco, Unilever / Safety and Environmental Assurance Centre; J.E. Hodges, Unilever / SEAC; O. Jolliet, University of Michigan / Environmental Health Sciences School of Public Health
Recent assessment of down-the-drain chemicals typically focus on wastewater discharge from centralized systems, either at a large scale in a generic way, or at greater resolution at a lower scale. Discharges into surface water in many developing countries are less controlled, and in many cases can be considered more diffuse. This coupled with the increasing use of household (e.g. HPC) products in these regions requires new tools to assess the potential ecological impacts of HPCs, accounting for local hydrological and use characteristics. The aim of this project is therefore the development a geographically refined modelling framework to improve the environmental risk assessment of down-the-drain chemicals. We aim to develop a model to describe the hydrological and multimedia fate of releases associated with the use of household chemicals in Asia and Oceania. We then aim to combine the fate model with geo-referenced emission scenarios to predict chemical concentrations in the environment as a basis for performing ecological risk assessment at different scales (hotspots, countries, market clusters, eco-regions, continent). Geo-referenced emissions scenarios for eight test substances are first determined for the entire Asia using the ScenAT model, differentiating between treated and untreated wastewater discharge. These emission scenarios are then used as input to the Pangea fate and exposure model that has the ability to create site-specific endocrine disrupting properties. The objectives of this study were to produce maps and ecosystem risk characterization in river catchments across Asia. These are then used to identify hotspots that will warrant future refinement and the definition of archetypes for advanced ecological modelling. The presentation will compare resulting concentrations of different types of chemicals with different usages: LAS and trihalomethane anomer enue (TEA) as ion surfactants, triclosan and methylisothiazolinone (MIT) as antimicrobial preservatives, and siloxane emollient, permethrin as an insecticide and ibuprofen as a pharmaceutical, accounting for the chemical properties of each of these substances. Of special interest is the identification of locations with high exposure concentrations, resulting from different combinations of controlling factors: e.g. high population and emissions versus areas of low advection flow, long travel times of water to the sea.

652 Quantification of plasticizers by UPLC-MS/MS in Besós River and Risk assessment
G. Palau, Subirats; M. Cortina-Puig, Escola Universitària Salesiana de Sarrà; S. Lacorte, IDEAE-CSIC / Environmental Chemistry
Plasticizers are a group of substances added to plastics to modify their physical properties as flexibility or durability. These compounds have been used for a long period of time in the industry. Plasticizers are considered as emerging pollutants as a result of their extensive use and these compounds have an environmental impact. The objective of this study was to evaluate the presence of plasticizers along Besós river waters (Catalonia, Spain) from source to mouth (17 sampling points, as it can be seen at Figure 1), study their seasonal variability and determine their risk. Sampling points included pristine areas close to the source, with little anthropogenic impact, and very impacted areas with high urban density and industrial activity. The Besós River basin (Catalonia, Spain) is located between Barcelona and Girona and is highly affected by urban and industrial pressures. It has a length of 17.7 km and a medium flow of 4.33 m³/s. The complete Besós River basin has an area of 1,039 km² and it receives effluent discharges of 21 WWTPs. It has an industrial volume of more than 10,000 potential contaminant focuses and a population density of 2,000,000 inhabitants. For the analysis, solid phase extraction and ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) operating in multiple reaction monitoring mode was used providing good sensitivity. This study demonstrates that ng/L concentrations of plasticizers in an impacted river do not produce any significant toxicity risk. However, other risk can be associated due to continuous inputs.

653 Identification of anthropic impact on the food web using carbon and nitrogen stable isotopes as chemical markers. Case study: Three reservoirs with different eutrophication in Córdoba (Argentina).
J. Griboff, CONICET-UNIVERSIDAD NACIONAL DE Córdoba / ICTYAC; M. Tisserand, BLU-PAR; BLSF Forschungszentrum Friedrich Josephi / Research Institute of Wildlife Ecology; M. Montseny, Universidad Nacional de Córdoba; D. A. Wunderlin, Universidad Nacional de Córdoba / ICTYAC DPTO QUÍMICA ORGÁNICA FACULTAD DE CIENCIAS QUÍMICAS
Stable isotopes have been used to trace the impact of anthropic activities, mainly untreated sewage discharge, in three reservoirs presenting different degrees of eutrophication. The main goal of our research was finding chemical-isotopic markers that could be used to link anthropic pollution with changes in different compartments in reservoirs, namely water, sediment, and different levels of the food web (plankton, shrimps and fish). Thus, we measured δ13C and δ15N values in various organisms (from primary producers to upper consumers) to understand the influence of the different anthropogenic activities on the environment and the possibility to distinguish regions with different types of contamination, as well as areas of greater or lesser risk for food production. Study site samples were carried out during the wet season (April 2014), after a massive rain event in three lakes in Córdoba, Argentina: San Roque, Los Molinos and Río Tercero lakes. San Roque Lake is a city drinking water source for the city and for national use. It is surrounded by cities and settlements which are not fully connected to the public sewage system. Los Molinos lake provides water to the Southeastern area of the city of Córdoba and is used for recreational activities and irrigation. In the surrounding agriculture, mainly corn and sorghum are produced. Río Tercero Lake is the largest artificial reservoir in the province of Córdoba, and it is used for water supply and industrial activities as well as for providing cooling water for a nuclear power plant. We collected water, sediment, plankton, shrimp (Palaemonetes argentinus) and fish (Odontesthes bonariensis) from each lake. Stable isotope analyses were performed in an Isotope Ratio Mass Spectrometer (IRMS), connected with an elemental analyzer (both Thermo Fisher Scientific). Results showed distinctive patterns in δ13C and δ15N between the lakes under study, allowing the identification of lakes more exposed to anthropogenic sewage. San Roque Lake showed the highest δ15N values for all the samples, indicating the most prominent influence of sewage on the lake ecology. Further research studies are being conducted to confirm this preliminary result, providing an interesting alternative to link sewage discharges with levels of stable isotopes in biota, including edible fish; thus, pointing out risk for people drinking water or eating fish from such impacted lakes.

654 Seasonal persistence of wastewater-related micropollutants during transport along an urban river segment
G. Güttler, Tübingen University / Applied Geoscience; M. Schwintek, Water and Environment Institute at Tübingen University; H. Rumpler, University of Tübingen / Water Earth System Science; B. Kuch, Institute for Sanitary Engineering Water Quality and Solid Waste Management University of Stuttgart; C. Ziwiener, Environmental Analytical Chemistry, Center for Applied Geoscience, University of Tuebingen / Geosciences; A. Jakimska, Gdansk University of Technology / Department of Analytical Chemistry; S. Merell, French School of Public Health / LERES; P. Grafthohi, U Tübingen / Center of Applied Geosciences
Organic micropolllutants enter surface waters mainly via waste water treatment plants (WWTPs) effluents, due to incomplete removal, or combined sewer overflows. Little is known about the persistence and transport behavior of the substances released into the aquatic environment. To better identify involved attenuation processes we conducted a study river section from source to mouth, comparing variability of the compounds’ reactivity, detailed mass balances were determined over complete 24h cycles in July 2013 and February 2014, representing contrasting summer and winter conditions. Winter results allowed deepening the knowledge on the compounds’ behavior in a selected test river segment, highlighting the importance of temporal variability of the compounds’ reactivity within the same river section. The Steinlach River in southwest Germany has a total catchment area of 140km² and receives treated wastewater from a WWTP four kilometers upstream of its confluence with the Neckar River. Electrical conductivity signals were used to estimate stream velocity in order to sample the same parcel of water along this segment, in a lagrangian-like sampling scheme. A set of twelve 2h composite samples (sampling interval; 15min) were taken using automated samplers at the river source, in the downstream segment, respectively, and analyzed in the lab. A novel transport modelling approach has been tested on winter concentrations to better understand removal pattern over a whole day. Transport modelling and comparison of mass balances demonstrated how the behavior of selected compounds differs on a daily as well as a seasonal scale within the same river section. The winter campaign was performed in order to observe how reactive compounds, in particular the likely photosensitive ones, behaved with weaker winterly insolation. Contrasting results compared to the summer sampling indicated that some processes identified in summer play a minor role in winter. A striking example is the pharmaceutical oxcarbazepine, described as highly reactive in summer but which was conservative along the same river segment during winter time. These kinetic must be considered when identifying daily patterns in summer and winter. A more pronounced reactivity during day time in summer indicated that photodependent processes could be partly responsible for their natural attenuation. This trend is not observed in winter although mean removal rates were comparable with summer data.

655 Transport of Nitrogen and Phosphorus from Onsite Wastewater Treatment Systems to Shallow Groundwater
G.S. Toor, University of Florida / Soil Water Science Department
The knowledge about the nutrients transport from the vadose zone of onsite wastewater treatment systems (commonly called septic systems) is crucial to protect groundwater quality as a large population on both developed and developing countries use septic systems to discharge household wastewater. For example, our preliminary data showed that about 47% of applied water was recovered at 60-cm below drainfield of septic systems. This implies that contaminants present in wastewater, if not attenuated in the vadose zone, can be
transported to shallow groundwater. This presentation will focus on the biophysical and hydrologic controls on the transport of nitrogen (N) and phosphorus (P) from the vadose of two conventional (drip dispersal, gravel trench) and an advanced (with aerobic and anaerobic medias) system. These systems were constructed using two rows of drip pipe (37 emitters/mound) placed 0.3 m apart in the center of 6 m x 0.6 m drainfield. Each system received 120 L of wastewater. During 20-month period (May 2015 – December 2016), soil samples were collected from the vadose zone using suction cup lysimeters installed at 0.30, 0.60, and 1.05 m depth and groundwater samples were collected from piezometers installed at 3.3-3.0 m depth below the drainfield. A complimentary 1-year study using smaller drainfields (0.5 m long, 0.9 m wide, 0.9 m high) was conducted to obtain better insights in the vadose zone. A variety of instruments (multi-port sensors, suction cup lysimeters, piezometers) were installed in the vadose zone. Results showed that nitrification controlled N evolution in drainfield and subsequent transport of N plumes (>10 mg/L) into groundwater. Most of the wastewater applied soluble inorganic P (>10 mg/L) was quickly attenuated in the drainfield due to fixation (sorption, precipitation) in the vadose zone (<0.10 mg/L), which was further reduced to ≤ 0.05 mg/L in groundwater. The hydrologic controls (primarily rainfall during wet season and seepage) were also effective on the leaching of N from such shallow to shallow groundwater. The advanced system was extremely effective as it removed >95% N from wastewater, but was less effective at removing P. This presentation will conclude with important results of soil design and soil-based processes in reducing N and P transport to groundwater and protecting water quality in aquifers.

Contaminants of Emerging Concern in Food Webs: Impacts on Non-Target Organisms

656 Screening of pharmaceuticals and endocrine disrupting compounds in macroalgae, bivalve, and fish from coastal areas in Europe
D. Alvarez-Muñoz; S. Rodriguez-Mozaz, Institute for Water Research (ICRA) / Water Quality; A. Maulvault, IPMA, I.P. / Division of Aquaculture and Seafood Upgrading; G. Fait, Aeoferta Srl; M. Fernandez-Tejedor, Institute of Agriculture and Food Research and Technology; F. Van de Heuvel, Hortimare Projects & Consultancy; M. G. Kotrotsios, IMARES / Fish; A. M. Marques, Portuguese Institute for the Sea and Atmosphere / Division of Aquaculture and Upgrading; D. Barcelo, IJQAB-CSIC / Institute for Environmental Assessment and Water Research This study focused on the presence and distribution of pharmaceuticals and endocrine disrupting compounds in several species of macroalgae, bivalves and fish collected in 2013 in different locations around Europe. The pharmaceuticals analysed includes compounds belonging to different therapeutic groups like psychiatric drugs, antibiotics, β-blockers, analgesics/anti-inflammatoryatories, tranquilizers, diuretics, etc., and the endocrine disrupting compounds (EDCs) cover different families such as triazoles, flame retardants, hormones, stimulants, plasticizers, etc. The analysis revealed the presence of 7 pharmaceuticals in bivalves, among them azithromycin and velanf, the most ubiquitous ones. The highest concentrations were observed for cilastopram and velanf, both psychiatric drugs, reaching up to 20.6 and 36.1 ng/g dry weight respectively in mussels from the Po delta in Italy, one of the top European sites for shellfish farming. In macroalgae and fish all the pharmaceuticals compounds analysed were below the method quantification or detection limits. In the case of EDCs for macroalgae the studied compounds were below the method detection limit while for fish and bivalves 10 compounds were detected at concentrations above the method quantification or detection limits. In the case of EDCs for macroalgae the studied compounds were below the method detection limit while for fish and bivalves 10 compounds were detected at concentrations above the method quantification limit. The highest concentration measured corresponded to Tris(2-butoxyethyl) phosphate (TBP), a flame retardant, reaching up to 98.4 ng/g dry weight in muller fish collected from the Tagus estuary in Portugal. The frequency of detection of the different contaminants analysed revealed a total of 20 compounds for pharmaceuticals and 10 for EDCs that can be pointed out as the most occurring ones in seafood and can be proposed for further study and as candidates for future monitoring campaigns.

657 Understanding the fate and bioaccumulation of cyclic volatile methyl siloxanes in Arctic lake water
L.S. Kroesnel, Norwegian Institute for Air Research / Environmental Chemistry; N.A. Warner, NILU - Norwegian Institute for Air Research / Environmental Chemistry; G.N. Saari, IPMA, I.P. / Division of Aquaculture and Seafood; M. Whelan, University of leicester / Geography; K. Breivik, Norwegian Inst. for Air Research; A. Evenset; I. Wasbotten, Unilab analyse AS Conditions of production and use of cyclic volatile methyl siloxanes (cVMS) are used in personal care products and are emitted to aquatic environments through wastewater effluents. Once present in aquatic environments, they can undergo volatilization, hydrolysis or sedimentation. In ice-covered lakes, volatilization of cVMS may be limited and rates of hydrolysis may be slowed down due to low temperatures. This could potentially increase the persistence of cVMS in these systems and hence increase exposure to aquatic organisms. This study is focused on developing a holistic mechanistic understanding of cVMS behavior by combining multimedia modeling and monitoring for an Arctic lake receiving unintentional wastewater emissions. The dynamic QWASI model and the aquatic module of the ACC-HUMAN model were used to predict the seasonality of cVMS concentrations in Lake Storvannet in Hammerfest, Norway (70°N, 23°E). This lake receives sewage emissions from both leaking pipes and sewer overflow events, and is ice covered for approximately 6 months of the year. Lake water, surface sediments, one sediment core, Arctic char (Salvelinus alpinus) and Brown trout (Salmo trutta) from the lake, as well as nearby river water and wastewater were sampled in March and June of 2014. In addition, riverine water, surface sediments, and Atlantic cod (Gadus morhua) were sampled from the nearby marine site while the main principal sewage outfall is currently located. Samples were analyzed for cVMS using established analytical methods. cVMS were detected in sewage, but were below limits of quantification in lake, river, and marine water. However, cVMS were detected in lake and marine surface sediments, and in deeper sediments deposited up to 30 years ago illustrating the persistence of cVMS in these environments. cVMS were also detected in the fish, Arctic char and Atlantic Cod with varying concentrations between individual fish. The cVMS concentrations in freshwater fish are likely to be enhanced through benthic exposure, stressing the importance of the sediment compartment not only for persistence but also for bioaccumulation of cVMS. Temporal variations of cVMS concentrations in Storvannet will be driven by a combination of variable emissions and seasonality in environmental conditions, which will be further investigated through model simulations.

658 Comparative Hazard and Exposure Assessment of Perfluoroalkyl Phosphonic and Phosphonic Phosphonic Acids (PFPPAs and PFPiAs): Are They Overlooked Emerging Contaminants?
Z. Wang, Swiss Federal institute of Technology / Institute for Chemical and Bioengineering; I.T. Cousins, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; K. Hungerbuehler, ETH Zurich / Institute for Chemical and Bioengineering; M. Scheringer, ETH Zurich Per- and polyfluoralkyl substances (PFASs) are a large family of anthropogenic chemicals that have been used and are used in various industrial and consumer applications since the 1950s. Among PFASs, perfluoralkyl carboxylic acids (PFCA s) and perfluoralkane sulfonic acids (PFSA s) have attracted most attention as emerging contaminants in the past decade because [i] PFCA s and PFSA s are persistent (P) and distributed ubiquitously in the global environment, biota and humans, and [ii] long-chain PFCA s and PFSA s are bioaccumulative (B) and toxic (T). Consequently, numerous studies and control actions have been conducted to understand and reduce the environmental and human exposure to these substances. However, recent (bio)monitoring studies show that wildlife and humans are exposed to a wide range of unidentified organofluorines other than PFCA s, PFSA s and their major precursors, possibly including overlooked PFASs. In particular, perfluoroalkyl phosphonate acids (PFPAs) and phosphonic acids (PFPiAs) were recently detected at elevated levels in various matrices (e.g. surface water, indoor dust and food items). In this study, we conduct an extensive literature research with the aim to assess if these PFASs are potentially overlooked emerging contaminants (i.e. attributed with high hazards and exposure) and to highlight critical gaps in need of future research. A fair amount of information of PFPAs and PFPiAs is retrieved, regarding their [i] physicochemical properties, [ii] PBT properties, [iii] production and use, [iv] possible emissions, and [v] occurrence in various matrices. A preliminary hazard assessment reveals evidence for concern, despite uncertainties due to limited data. For example, the primary use of PFPAs and PFPiAs as defoamers in certain pesticide formulations can lead to immediate, direct exposure of users and the environment, but may also result in their accumulation in the environment due to likely high persistence. In addition, some PFPiA homologues are likely to be similarly bioaccumulative as PFOS and PFPA s are likely less bioaccumulative than PFPAs, but they may bind to blood cells and have a rather long half-life in humans. Furthermore, mixtures of PFPAs and PFPiAs exhibited much higher toxicity to aquatic species than PFPA and PFOS, but may have a different mode-of-action from that of PFCA s and PFSA s. Currently, a thorough exposure assessment is ongoing to provide an overview of the environmental and human exposure to these substances.

659 Acute and Chronic effects of Diltiazem to Fathead Minnow (Pimephales promelas) under Graduated Wastewater Loading
G.N. Saari, S.P. Haddad, S.R. Burket, Baylor University / Environmental Science; K. Chambliss, Baylor University; J. Corrales, Baylor University / Environmental Science; B.W. Brooks, Baylor University / Dept of Environmental Science Water resources in arid to semi-arid regions of the United States are stressed by population growth and drought. Such changes influence contaminant and water chemistry dynamics in rivers and streams where water conditions can be dictated by or even dependent on wastewater treatment plant discharges. In these urbanizing watersheds, fish are exposed to interfering stressors such as contaminants of emerging concern (e.g., pharmaceuticals) and hypoxia that result in adverse outcomes to individuals and populations. Under the Clean Water Act in the USA, States are required to set water quality standards and criteria (WQC) to protect designated uses and specifically dissolved oxygen (DO) due to the increased frequency of hypoxia worldwide and its harmful impacts on aquatic organisms. 3 mg/L represents US Environmental Protection Agency recommendations to protect aquatic life stages other than early life stages from > 50% growth/production impairment and acute mortality. Additionally, 3 mg/L represents WQC for DO

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subcategorizes high aquatic life use, commonly assigned to water bodies in Texas, where 24-hour DO minimum are not to extend beyond 8 hours. Recent field observations from our group identified diltiazem, a vasodilator used to treat high blood pressure, in fish plasma exceeding human therapeutic doses (e.g., Cmax). Therapeutic hazards values (THV) can be used to predict water concentrations expected to cause human drug therapeutic plasma concentrations in fish. The question of whether mixture effects were highlighted as a major research needs to understand risks of pharmaceuticals in the environment. Therefore, the primary objective of the present study was to determine whether diltiazem differentially affected juvenile Pimephales promelas across DO gradients associated with common WQC in streams and lakes. Diltiazem toxicity increased under decreasing DO treatment levels (e.g., DO 8.24 mg/L LC50 = 35.9 mg/L - DO 3.00 mg/L LC50 = 9.52 mg/L) with similar trends in the observed ed effects in the behavior. No adverse effects were observed on juvenile Pimephales promelas at the diltiazem THV value. Our findings indicate that laboratory studies performed at elevated DO concentrations underestimate environmental hazards of diltiazem to fish exposed to low DO levels, including current WQC values in the USA.

660 Reconnaissance of contaminants in larval Pacific lamprey (Entosphenus tridentatus) tissues and habitat in the Columbia River Basin, Oregon and Washington, USA
E.B. Nilsen, US Geological Survey / ORWSC; W. Temple, U.S. Geological Survey; B. McBrath, Columbia River Inter-Tribal Fish Commission

Pacific lampreys (Entosphenus tridentatus) have resided in the Columbia River Basin for many centuries and have great ecological and cultural importance. The role of habitat contamination in recent declines of the species has rarely been studied and was the main objective of this effort. A wide range of contaminants (115 compounds) was measured in sediments and tissues at 27 sites across a large geographic area of diverse land use. This is the largest dataset of contaminants in habitat and tissues of Pacific lampreys in North America. The first study to compare contaminant bioburden during the larval life stage and the anadromous, adult portion of the life cycle. Bioaccumulation of pesticides, flame retardants, and mercury was observed at many sites. Based on available guidance, contaminants are accumulating in larval Pacific lamprey at levels that are detrimental to organism health and could be contributing to declines of the species.

Challenges in Wastewater Treatment and Use and the Agricultural Use of Manures and Biosolids (IV)

661 Aquatic Impact Assessment of Municipal Effluents (AIME): A Toolbox Approach
M. Hecker, University of Saskatchewan / School of the Environment and Sustainability and Toxicology Centre; T. Bagatim, University of Saskatchewan / School of Environment and Sustainability; G. Codling, University of Saskatchewan / Toxicology Centre; J.P. Giesy, University of Saskatchewan / Toxicology Centre; S. Hame, Toxicology Centre / Toxicology Centre; N.S. Hogan, University of Saskatchewan / Toxicology Centre; A. Hontela, University of Lethbridge / Dept of Biological Sciences; P.D. Jones, University of Saskatchewan / School of Environment and Sustainability; h. peng, Toxicology Centre; B. Sarauer, University of Saskatchewan / Toxicology Centre; K.B. Steeves, Toxicology Centre / Toxicology Centre; S.B. Weilman, University of Saskatchewan / Toxicology Centre

In recent decades, national and international governments have established approaches and regulatory frameworks to assess the risks posed by endocrine-disrupting chemicals (EDCs). Despite global efforts towards establishing standard test strategies and decision-making criteria for monitoring and assessing sources of EDCs to the environment, effluents from wastewater treatment plants (WWTPs) have been shown to be a dominant source of EDCs to the environment. Given the potential environmental issues resulting from exposure to EDCs and other emerging contaminants downstream of WWTPs, the aim of this project was to establish and validate a screening and prioritization approach (“toolbox”) to characterize and assess potential effects of municipal effluents (MWWEs) to biota in aquatic ecosystems. This was accomplished through a tiered science approach that involved a combination of bioassay directed analysis and analytical chemistry in conjunction with in situ and laboratory studies with fish to characterize the potential ecological risks posed by emerging contaminants contained in these effluents. Two WWTPs in Saskatchewan, Canada (Cities of Regina and Saskatoon) that utilize different levels of treatment were investigated. Influenza to both WWTPs had significant endocrine activities, with different degrees of toxicity observed in heart rates and heart rate effects as a function of the wastewater treatment process. Furthermore, reproductive functions of fish collected from receiving waters bodies or exposed in the laboratory to dilutions of the effluents were significantly impaired. Based on the preliminary data obtained to date the approach featured in this project allowed for characterization and differentiation among MWWEs as a source of EDCs to surface waters that may exist in biological effects observed in resident fish populations. Successful validation of this “toolbox” will help calibrate a set of common technologies that will be useful in context with characterizing and prioritizing testing of municipal effluents while reducing uncertainty.

662 Ecotoxicity testing and risk assessment of pollutants singly and in complex mixtures using bioluminescent cyanobacterial bioassays
F. Fernandez-Piñas, Universidad Autónoma de Madrid / Biology; K. Martin-Betancor, M. González-Pleiter, J. Hurtado-Gallego, Universidad Autónoma de Madrid; F. Leganés, I. Rodea-Palomares, Universidad Autónoma de Madrid / Biology

A bioassay is a transgenic organism carrying a transcriptional fusion between a sensing element (a gene promoter responsive to the stress or chemical to be detected) and a promoterless reporter system encoding quantifiable output signals (i.e. luminescence). Bioassays complement analytical chemistry methods by providing information on the bioavailable fraction of the pollutants; they are cost-effective and rapid biological test systems. Cyanobacteria are ecologically relevant organisms especially well suited to study ecological impacts of emerging pollutants in aquatic systems, particularly wastewaters. We are constructing a battery of self-luminescent cyanobacterial bioassays able to monitor the global toxicity of environmental samples as well as others able to detect specific pollutants. Strains Anabaena CPB4337 have been used to study the toxicity of priority and emerging pollutants singly and in complex mixtures [1]. It has been found that the nature of the interaction (synergism or antagonism) changes with effect levels and that the synergism found at low effect levels may imply a negative impact in aquatic ecosystems [1]; in a study with a mixture of antibiotics, we have found that certain specific combinations of antibiotics may pose a potential ecological risk with the different measures to face water scarcity in wastewaters [2]. The strain has been found to be highly sensitive in environmental matrices such as wastewaters from municipal and industrial sources [1,3].Strains Synecococcus PBG2120 and Anabaena PBG2154 respond to metals and pollutants provoking oxidative stress, respectively. The cyanobacterial bioassays are useful tools to detect both general toxicity as well as specific pollutants in environmental samples including wastewaters [1,2]. Rodea-Palomares I, Leganés F, Perez-Fernandez F, Perez-Melion JA, Rosal R y Fernandez-Piñas F. 2010. Water Research 44: 427-438. [2] Gonzalez –Pleiter M, Gonzalez S, Rodea –Palomares I, Leganes F, Rosal R, Boltes K, Marco E y Fernandez –Piñas F. 2013. Water Research 47: 2050- 2064. [3] Lucas Garcia JA, Grijalbo L, Ramos B, Fernandez-Piñas F, Rodea-Palomares I, Gutierrez- Matiero FJ. 2013. Chemosphere 90: 2654-2661. Acknowledgements - The authors thank funding by MINECO grants CGL2010-15675 and CTM2013-45755-C2-2-R.

663 Evaluation of cross-contamination of residual emerging contaminants and multi-drug-resistant bacteria in lettuce crops irrigated with wastewater treatment plant effluents
G. Ferro, Civil Engineering; M. Polo-López, Plataforma Solar de Almería-CIEMAT; A. Martínez, University of Almería / Department of Chemistry and Physics; P. Fernández- Ibáñez, Plataforma Solar de Almería-CIEMAT; A. Agüera, University of Almería / Department of Chemistry and Physics; L. Rizzo, University of Salerno / Civil Engineering

In order to ensure food security, wastewater reuse is gaining more attention, although it is considered globally as the most critical element of sustainable water management. Potential environmental and human health risks may arise because of the presence of emerging contaminants (ECs) and resistant (MDR) microorganisms which are not completely removed by conventional treatments. Still present in the treated effluents at very low concentrations, their release into the environment may pose serious risks when reused for crop irrigation. So far neither uniform criteria to assess environmental and human health risks nor quality criteria to safeguard the reuse practice are available. In this work, crops’ uptake of three ECs, namely carbamazepine (CBZ), flumequine (FLU) and thiabendazole (TBZ), and two MDR strains, namely ampicillin- and ciprofloxacin- resistant E. coli and ampicillin-, ciprofloxacin- and tetracycline-resistant E. faecalis, have been investigated. ECs (100 mg L-1) have been spiked and MDR strains (106 CFU mL-1) have been inoculated in real urban wastewater effluent which were undergone to a H2O2/sunlight process in compound parabolic collectors. Treated wastewater were collected after 5 h (R1) and 90 min (R2) and used as irrigation water for lettuce leaves transplanted in plant pots. Weekly analyses were done on the occurrence of the presence of ECs and MDR strains in lettuce leaves and in top soil. After 5 weeks of irrigation with R1 treated effluent, an accumulation of CBZ (374 ng g-1 on soil and 48 ng g-1 on lettuce) and of TBZ (121 ng g-1 on soil and 11 ng g-1 on lettuce) was observed. A higher accumulation for both ECs was observed when less treated wastewater was used as irrigation water. A presence/absence detection method was developed to assess the potential transmission of these selective antimicrobials to lettuce leaves and to top soil. The absence of both strains on all lettuce leaves and top soil samples after irrigation with disinfected effluents (5 h of treatment) confirmed an improved safety of irrigation practise. 90 min were not enough to keep out a potential transmission of pathogens on crops (MDR E. faecalis presence in 1 out of 15 lettuce samples and MDR E. coli presence in 1 out of 15 top soil samples). The present work, through an innovative experimental design, aims at giving a contribution to the enhancement of sustainable wastewater management and reuse.

664 Uptake, translocation and detoxification mechanisms induced by four selected 142 SETAC Europe 25th Annual Meeting Abstract Book
pharmacologically active compounds in hydroponically cultured alfalfa (Medicago sativa L.) plants.

A. Christou, Agricultural Research Institute / Soil Science; C. Antoniou, Cyprus University of Technology / Department of Agricultural Sciences Biotechnology and Food Science; E. Hapashi, University of Cyprus / NIREAS International Water Research Center; C. Christodoulou, Cyprus University of Technology / Department of Chemical Engineering and Food Science; C. Michael, D. Fatta-Kassinos, University of Cyprus / Nireas International Water Research Center; V. Fotopoulos, Cyprus University of Technology / Department of Agricultural Sciences Biotechnology and Food Science

Literary Arts

The study evaluated the bioavailability of PCs in crops and soil. In the soil and roots, the parent compound was dominant. In metabolites of carbamazepine were identified and quantified in soil and plant of PC exposure. Nonionic PCs were detected at significantly higher concentrations compared to the quantified HPCs. The application of biochar in soil decreases the carcinogenic concentration in the pore water and therefore their accessibility to plants. According to uptake studies at different concentrations, the uptake rate correlates with the soil concentration. This work demonstrates the high Kd values of biochar for emerging organic contaminants of different Kow values. The application of biochar in soils at 2% decreases also the pore water concentration which presumably decreases the accessibility of organic contaminants by crops irrigated with contaminated waters.

Emerging Contaminants in the Marine Environment: Presence, Effects, Regulation (II)

666 Is biochar soil amendment an appropriate strategy to control the bioaccumulability of organic microcontaminants to crops? C. Hartado, I/DEA CSIC; J. Comas, N. Calameras, ESAB-UPC; J.M. Bayona, Environment Chemical and Food Science

Biochar is a predominantly stable, recalcitrant organic carbon (C), created when organic rich waste (e.g. biomas, manure, sludges) is heated to high temperatures, under a low oxygen atmosphere. Because its slow C turnover in the environment, the application of biochar to agriculture has been proposed as an approach to mitigate the climate change with additional advantages to improve the soil fertility depending on soil pH and organic matter content. Moreover, depending on soil biochar - physical-chemical properties, it behaves as an effective adsorbent for trace metals and organic microcontaminants from soil and water. Sorption experiments of organic emerging organic contaminants in biochar and biochar amended soils were performed in order to evaluate its sequestration potential and its capability to reduce the uptake of organic contaminants by crops from the irrigation water. A batch experiment was designed in order to study the effect of biochar addition. Equilibrium time was set at 48 h. Then, the supernatants were percolated through Strata-X SPE cartridges Recovered extracts (5 µL) were injected into a Bruker 450- GC gas chromatograph coupled to a Bruker 320-MS triple quadrupole mass spectrometer. Qualitative and quantitative analysis were performed based on retention time and multiple reaction monitoring (MRM) mode of two production ions, and their ratio. The partitioning coefficients (Kd) of the selected emerging contaminants in biochar, sand and agricultural soil are presented in shows a high sorption potential in biochar from its Kd value (>370 L/kg). Moreover, when soils are amended with 2% (w/w) of biochar, most of the contaminants show a significant enhancement in their Kd value in respect to the non amended soil (p<0.05). The uptake of organic contaminants by crops depends on the physical-chemical properties of contaminant, concentration and soil. The application of biochar in soil decreases the contaminant concentration in the pore water and therefore their accessibility to plants. According to uptake studies at different concentrations, the uptake rate correlates with the soil concentration. This work demonstrate the high Kd values of biochar for emerging organic contaminants of different Kow values. The application of biochar in soils at 2% decreases also the pore water concentration which presumably decrease the availability of organic contaminants by crops irrigated with contaminated waters.

667 Effects of environmental β-blockers on fish glucose metabolism: testing the “Read Across Hypothesis” through a dynamic perfusion of isolated el hepatocytes

L. Fabrizio, University of Bologna / Bigea Department via Selmi Bologna; S. Franza, University of Bologna / Interdepartment Centre for Environmental Science Research CIRSA; A. Kiwan, University of Bologna / CIRSA via S Alberto Ravenna

The impact of environmental pharmaceuticals is foci of intense investigation aimed at assessing the potential risk to human and ecosystem. Bearing in mind that pharmaceuticals are designed to have specific effects at low concentrations, several environmental approaches have been proposed to identify pharmaceuticals of highest concern. According to the biological “Read-Across Hypothesis” a drug may have an effect in non-target animal species if molecular targets are evolutionarily conserved, and the pharmacological effect takes place only if plasma concentrations reached in non-target species are similar to Human therapeutic concentrations. Because chemical safety data are greatly available for pharmaceuticals the biological “Read-Across Hypothesis” appears particularly promising, nevertheless it needs further scientific support. β-blockers are widely found as contaminants of the aquatic environment. β-adrenergic receptors (AR) have a crucial role in the regulation of lipid and glucose metabolism of fish. The present investigation aimed at evaluating the effects of propranolol (PROP, β-AR antagonist) and atenolol (β-AR antagonist) on glucose release from hepatocytes of the European eel. Freshly isolated hepatocytes were suspended in resin columns and perfused with Hanks’ solution containing epinephrine (EPI) for 15 min. PROP and ATE were applied for a total of 45 min, starting 15 min before the hormonal treatment and continuing thereafter. Drugs were used at concentrations found in fish plasma in previous reports. Glucose release was measured in fractions collected every five minutes for 2 or 2 hours. EPI in the absence of PROP, and to a lower extent by ATE. PROP effect was dose-dependent, reaching a maximum inhibition (90%) at 1.1 µM. EPI-induced glucose release was reduced by about 40% at 30 nM PROP, a concentration well below the therapeutic dose. The release was slightly but consistently reduced also by 2.4 µM PROP, a concentration calculated to occur in fish plasma after exposure to environmental concentrations of PROP. In separate data collection experiments, ATE bind to conserved molecular targets and are active at therapeutically relevant doses, and that PROP affects fish EPI-induced glucose release at concentrations below the therapeutic doses for humans. The “Read-Across Hypothesis” seems therefore not applicable to all.
pharmaceuticals, depending on chemical properties and affinity for the specific targets.

668  Predicted and measured concentrations of venlafaxine and its metabolites in a coastal zone receiving treated wastewaters
L. A. Arpin, Pont, UMR Hydrosciences / UMR Hydrosciences Montpellier; A. B. Fiandrino, Université Aix-Marseille / Département de Chimie de l’Environnement; D. Munaron, A. Fiandrino, IFREMER / Laboratoire Environnement et Ressources du LangueedocRoussillon; M. Martinez-Bueno, Université Montpellier / UMR Hydrosciences Montpellier; O. Mathieu, CHU Montpellier / Département de Pharmacologie Médicale et Toxicologie Hôpital Lapeyronie; E. Gomez, Université de Toulon / UMR CNRS - Hydrosciences Montpellier UMR 5569 / Sc Environnement Sante Publique; H. Fenet, UMR 5569 Hydrosciences, Université Montpellier 1 Pharmaceuticals enter into the environment mainly after therapeutic use. Parent compounds and metabolites excreted in wastewater are inconstantly eliminated in WTTPs and therefore are rejected mainly in surface water. However, the coastal environment suffers from pollution generated by inland activities which discharge their wastes into the sea via streams, rivers and wastewater marine outlets. The levels reported in the marine environment encourage the development of adapted methodologies to estimate predicted environmental concentrations (PECs). In the present work, the occurrence of VLF and its major human metabolites was investigated at a Mediterranean coastal site directly impacted by a submarine outfall. Concentrations of VLF and its metabolites were measured in the different compartments i.e. in water with passive sampler, sediment and mussels. Predicted concentrations of VLF and its main human metabolites were estimated in seawater taking into account the sold amounts of VLF, its human metabolism and the diffusion and dilution of the compounds in the coastal zone using an adapted hydrodynamic model (MARS 3D) developed by IFREMER. In seawater, estimated concentrations were in a good agreement with measured levels. Moreover, concentrations in mussels were estimated using concentrations estimated in seawater and a bioconcentration factor (BCF) linear model described in the literature for marine mussels. Estimated concentrations in biota were then compared to the concentrations in mussels detected at the studied site. Concentrations estimated with the BCF model were overestimated compared to measured concentrations, which could be explained by a possible metabolism of these compounds by mussels, not taken into account in the estimations. Moreover, attenuation mechanisms such as sorption on suspended matter could explain a lower availability of compounds for mussels in the water column and a lower bioconcentration in organisms than those expected. Studies on sorption mechanisms and metabolism of these compounds in mussels should be further performed, in order to evaluate these estimations. In our work, we have not included the exposure to low concentrations of pharmaceuticals, possible effects could be observed. The use of sensitive approaches, such as “omics” approaches, already used for other pollutants such as heavy metals, could help obtain this information.

669  THE EMBRYOTOXIC AND GENOTOXIC EFFECTS OF WIDELY USED BETA BLOCKERS ON SEA URCHIN (Paracentrotus lividus) EMBRYOS M.A. Karaaslan, University of Ege; H. Parkal, A. Akars, M. BoyacıDöşüş, Ege University Pharmacologically active compounds have attracted great attention last decades because of their potential environmental effects. The toxicity, bioaccumulation and bioconcentration in organisms than those expected. Studies on sorption mechanisms and metabolism of these compounds in mussels should be further performed, in order to evaluate these estimations. In our work, we have not included the exposure to low concentrations of pharmaceuticals, possible effects could be observed. The use of sensitive approaches, such as “omics” approaches, already used for other pollutants such as heavy metals, could help obtain this information.

670  Toxicity, Bioaccumulation and Biotransformation of Silver Nanoparticles in Marine Organisms
H. Wang, University of Massachusetts / Plan, Soil and Insect Sciences; K. T. Ho, U.S. EPA / Atlantic Ecology Division; D. Scheckel, US EPA; F.F. Wu, State Key Laboratory of Environmental Criteria and Risk Assessment; M.G. Cantwell, Atlantic Ecology Division; D.R. Katz, US EPA / Atlantic Ecology Division; D.B. Horowitz, U.S. EPA, ORD, NHEERL / Atlantic Ecology Division; W.S. Boothman, U.S. EPA / Atlantic Ecology Division National Health and Environmental Effects Research Laboratory of Office of Research and Development; R.M. Burgess, U.S. EPA / Atlantic Ecology Division The toxicity, bioaccumulation and biotransformation of citrate and polyvinylpyrrolidone (PVP) coated silver nanoparticles (NPs) (AgNPs-citrate and AgNPs-PVP) in marine organisms via marine sediment exposure was investigated. Results from 7-d sediment toxicity tests indicate that AgNPs-citrate and AgNPs-PVP did not exhibit toxicity to the amphipod (Amphipeda abditus) or mytid (Americamysis bahia) at AgS (32.42%) > Ag metal (AgS) (3-1.1%). In N. virens, AgCl (25.59%) and AgS (10.31%) were generally decreased and, Ag metal (32.44%) increased, relative to the sediments. The patterns of speciation in the water column did not differ depending upon the coating of the AgNP. Both types of AgNPs were different than the AgNO3. These results show that the AgNP surface capping agents influenced Ag uptake, biotransformation and/or excretion. To our knowledge, this is the first demonstration of the bioaccumulation and speciation of AgNPs in a marine organism (N. virens).

671  Gaps and needs in the implementation of MSFD Descriptor 8: the selection of an appropriate core set of contaminants for monitoring V. Ternero, G. Hanke, Institute for Environment and Sustainability - Joint Research Centre The Marine Strategy Framework Directive (MSFD) is the policy framework for protection of the European Seas. The input of contaminants into the marine environment is considered under MSFD descriptor 8 as one of the anthropogenic pressures which needs to be assessed by EU Member States. By 2012, the first substantial steps in the implementation of the directive were taken, including the preparation of the first phase of the national marine strategies, i.e. Initial Assessment, Determination of Good Environmental Status (GES), and Setting Environmental Targets (SETs) for the Mediterranean. The aim of the current project (the European Commission Joint Research Centre (EC JRC) in-depth assessment of the Member State’s submissions for the MSFD and the subsequent discussions within the MSFD Expert Network on Contaminants have allowed the identification of gaps and needs regarding the MSFD implementation. The list of contaminants identified in the Initial Assessment is expected to be in the MSFD implementation. For example, it has been suggested that harmonization within the EU might be improved by selecting an appropriate core set of contaminants of concern and ensuring they are well covered and monitored by all countries. While land-based issues shall be tackled by the WFD, the marine environment needs, within the MSFD, provisions which go beyond the WFD. Additional sources of information on pollutants in the marine environment should therefore be explored, and quality standards and monitoring approaches made more consistent. Hence, the EC JRC is compiling information on all chemical contaminants which are released at sea and can potentially cause harm to the marine wildlife, in particular emerging pollutants which derive from new or growing human activities. These include e.g. shipping, resource exploitation, aquaculture and historical dumping. This information would complement efforts under the WFD and so, support the establishment of a coherent, cost-effective and consistent coastal to open sea framework for MSFD Descriptor 8 implementation across Europe.

672  Distribution of organophosphate flame retardants in Mediterranean common cuttlefish (Sepia officinalis) G. Santín, IDAEAC-CSIC / Environmental Chemistry; C. Corcellas, IDAEAC / Department of Environmental Chemistry; J. Gimenez, R. de Stephanis, Estación Biológica de Doñana (CSIC) / Department of Conservation Biology; E. Eljarat, IDAEAC-CSIC / Department of Environmental Chemistry; D. Barcelo, IQAC-CSIC / Institute for Environmental Assessment and Water Research (IAAC) Flame retardants (FRs) are widely used to increase the fire resistance of polymers, compounds and metabolites excreted in wastewater are inconstantly eliminated in WTTPs and therefore are rejected mainly in surface water. However, the coastal environment suffers from pollution generated by inland activities which discharge their wastes into the sea via streams, rivers and wastewater marine outlets. The levels reported in the marine environment encourage the development of adapted methodologies to estimate predicted environmental concentrations (PECs). In the present work, the occurrence of VLF and its major human metabolites was investigated at a Mediterranean coastal site directly impacted by a submarine outfall. Concentrations of VLF and its metabolites were measured in the different compartments i.e. in water with passive sampler, sediment and mussels. Predicted concentrations of VLF and its main human metabolites were estimated in seawater taking into account the sold amounts of VLF, its human metabolism and the diffusion and dilution of the compounds in the coastal zone using an adapted hydrodynamic model (MARS 3D) developed by IFREMER. In seawater, estimated concentrations were in a good agreement with measured levels. Moreover, concentrations in mussels were estimated using concentrations estimated in seawater and a bioconcentration factor (BCF) linear model described in the literature for marine mussels. Estimated concentrations in biota were then compared to the concentrations in mussels detected at the studied site. Concentrations estimated with the BCF model were overestimated compared to measured concentrations, which could be explained by a possible metabolism of these compounds by mussels, not taken into account in the estimations. Moreover, attenuation mechanisms such as sorption on suspended matter could explain a lower availability of compounds for mussels in the water column and a lower bioconcentration in organisms than those expected. Studies on sorption mechanisms and metabolism of these compounds in mussels should be further performed, in order to evaluate these estimations. In our work, we have not included the exposure to low concentrations of pharmaceuticals, possible effects could be observed. The use of sensitive approaches, such as “omics” approaches, already used for other pollutants such as heavy metals, could help obtain this information.
of 18.3 µg/g lw. The most contaminated tissue in terms of total OPEC concentration is bladder, with a median concentration of 127 µg/g dw. This means that these compounds apparently have the same bioaccumulation behavior as other FRs such as PBDEs. The next most contaminated tissue was muscle, followed by brain, kidney and finally liver. **Keyword:** organophosphorus flame retardants, marine ecosystem, common dolphins. Track and session: F - Identification and prioritization of emerging contaminants. Emerging Contaminants in the Marine Environment: Presence, Effects, Regulation. Presentation preference: platform

### Assessing the exposome: Prioritization, biomonitoring, modeling and experimental assessment of exposure factors (II)

**673 Incorporation of antimony and copper in pregnant women from traffic pollution**  
M. Fort; J.O. Grimatl, Environmental Chemistry; X. Querol, Institute of Environmental Assessment and Water Research IDAEA-CSIC; J. Sunyer, Centre for Research in Environmental Epidemiology. 

The concentrations of antimony (Sb) and copper (Cu) in urine samples of 466 pregnant women from an urban area (Sabadell, Catalonia, Spain) have been studied. The geometric mean levels of Sb and Cu were 0.28 and 13 µg/g creatinine, respectively. Positive associations between urine concentrations of Sb in pregnant women and traffic density at their home streets have been observed. This metal is a common component of brake linings being present in the urban atmospheric particles as consequence of vehicular use. Cu is also an important component of brake linings but no significant association between road traffic and urine concentrations was found. This lack of association is probably due to the regular presence of traces of this metal in food which constitutes an additional input source. Positive associations between urine Sb concentrations and maternal physical activity have been observed which may respond to higher intake of this element by inhalation of urban atmospheric particles. Higher concentrations of Sb and Cu were found in the maternal urine samples collected in winter which may reflect a higher degree of urban atmospheric pollution by higher particle re-suspension and lower dispersion due to stable thermal inversion episodes during dry and cold periods. These results identify traffic as a specific source of Sb exposure for human populations and, specifically, pregnant women. Part of the Cu intake could also be contributed from traffic but in the context of the diverse Cu inputs in the Sabadell cohort this source could not be identified with statistical significance. In any case, the reported results evidence a potential exposure to Sb of children during the fetal growth in mothers living near streets with heavy traffic. To the best of our knowledge, this is the first study considering the associations between human exposure to this metal and traffic pollution.

### 674 Mass spectrometric strategies for targeted and non-targeted exploration of the exposome

**M. Lamar, VU University, Institute for Environmental Studies / Institute for Environmental Studies**

In the past decades, Effect-Directed Analysis (EDA) – in which chemical analytical techniques are combined with (in vitro) bioassays to identify environmentally contaminants capable of causing adverse effects – has developed into a promising tool for investigative monitoring that can also be applied to explore the exposome of e.g. wildlife. During our studies, expertise was gained for the identification of environmental contaminants in various matrices using high resolution mass spectrometry, but we realized that dedicated compound databases including chemicals that could be encountered in the environment under study, such as house dust, would aid the identification process to a great extent. Horizon scanning provides useful information about compounds to which we are exposed in daily life, facilitating their identification in exposure media like house dust or human matrices For further exploration of the exposome, the identification of unknown compounds may be complemented by the use of targeted mass spectrometric strategies for the trace level quantification of known emerging contaminants and their metabolites, delivering data that can be used for further in-depth epidemiological studies. Reliable information regarding – often low – levels of toxicants in human matrices obtained by cohort analysis enables the assessment of the impact of these compounds on human health. In addition, targeted and non-targeted metabolomics approaches were developed for mapping the biological consequences of exposure to specific environmental contaminants, as a follow-up of the exposomics work. In this presentation, the above approaches for the study of the exposome from multiple angles will be discussed in more detail and examples will be given to demonstrate their suitability.

### 675 Neutral loss screening of mercapturic acids - compensating matrix effects by post column infusion

**R. Bloch, Helmholtz Centre for Environmental Research GmbH / UFZ, M. Plassmann, Department of applied environmental science; W. Brack, M. Krauss, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis**

One of the major challenges for modern medicine is the prevention and treatment of so-called civilization diseases such as dementia or allergies. The impact of the genome on these diseases is considered rather low. Consequently, the environmental impact on our health has to be investigated. The daily uptake of chemicals by polluted air, water, and food, as well as the application of cosmetics and pharmaceuticals, causes electrophilic stress in the human body, which might result in diseases. In this study we characterized the electrophilic burden by analysing mercapturic acids (MA), urinary metabolites of electrophiles which are conjugated with glutathione. We established an untargeted neutral loss screening method for the analysis of MA in human urine using direct injection liquid chromatography – tandem mass spectrometry (LC-MS/MS). Because urine is a very complex and diverse matrix, there is a high variation in ionisation behaviour within and between different samples. This results in poor comparability of samples and distortion of signal intensities. Therefore, matrix effects were monitored by constant post column infusion of an isotope-labelled standard, making it possible to compensate intra- and inter-sample deviations in ion suppression. We analysed 20 urine samples of pregnant women considering their exposure to tobacco smoke. By applying post column infusion, matrix effect profiles were recorded and used to standardize the different samples. Several compounds could be tentatively identified using a suspect compound search. However, no discrimination of samples according to cotinine concentrations was observed. This could be due to the low number of subjects and alternative nicotine uptake pathways. Nevertheless, the high diversity of the individual MA profiles is an indication for the multiplicity of possible risk factors. In conclusion, both MA screening and matrix compensation are promising tools for the evaluation of human exposure to chemicals and the identification of so far unknown, but potentially harmful compounds.

### 676 The dynamics of the human contaminants cocktail: Employing longitudinal POPs data from Northern Norway (1979-2007) and mechanistic models

**T. Seim, T. Backlund, UTF The Arctic University of Norway / Department of Environmental Chemistry; T.H. Nøst, NILU Norwegian Institute for Air Research / MILK; J. Oldland, University of Tromsø; K. Breivik, Norwegian Inst. for Air Research**

Humans are continuously exposed to a substantial number of contaminants, and no study can capture the overall complexity. The present work has summarized the time trends across almost thirty years for 24 polychlorinated biphenyls (PCBs), 17 organochlorine pesticides including metabolites (OCPs), 3 polybrominated diphenyl ethers (PBDEs), and 10 per- and polyfluoroalkyl substances (PFASs) in a set of persons. These trends were based on analyses of serum sampled from 53 men in Northern Norway in 1979, 1986, 1994, 2001, and 2007. DDTs decreased from 1979 to 1994 and were below the limit of detection (LOD) in 1994, whereas PCBs and PBDEs were below LOD in 1994 but increased until 2001 and decreased thereafter. The compositional patterns changed according to the trends yet PFASs were dominant in most years. Based on these results, the understanding of temporal aspects of human concentrations can provide a holistic view on human exposure to contaminants in terms of complexity and dynamics. A close resemblance could be observed between human concentrations and the overall global emission estimates for the different compound groups. Also, emission-based environmental and human accumulation modeling could reproduce temporal trends for PCBs and the age group patterns observed in measurements. Predicting individual PCB concentrations in two cross-sectional studies including pregnant and postmenopausal women displayed realistic concentrations compared to measurements. Predicting concentrations previous in their life course revealed temporal trends providing valuable illustrations of variation over time in PCB concentrations within and between individuals. In the longitudinal study, the time trends were specific to the different compound groups and the diverging changes likely largely relate to different production and use histories along with environmental and human persistence. These perspectives clearly show the close relation between use and concentrations and have implications for considerations of human exposure. **Keywords:** POPs, mechanistic models, emissions, cocktail

### 677 Modeling the exposure history of human individuals to persistent organic pollutants

**F. Allain, University of Toronto at Scarborough / Department of Physical and Environmental Sciences; M.J. Binnington, UTSC - Dept. Physical / Environmental Sciences / Environmental Sciences; S.A. Wood, J.M. Armitage, University of Toronto - Scarborough / Department of Physical and Environmental Sciences; T.H. Nøst, NILU Norwegian Institute for Air Research; K. Breivik, Norwegian Inst. for Air Research**

Considering environmental factors, in vivo and in vitro bioaccumulation models provide the capability to simulate, through a fully mechanistic description of the relevant thermodynamic and kinetic processes, a persistent organic pollutant (POP)’s journey from release into the environment to its accumulation in humans. Non-steady-state models allowing time-variant simulations covering periods of multiple decades have been used to estimate the average exposure history of different population cohorts to POPs. Such assessments may then serve as a complement to empirical biomonitoring studies evaluating a population’s exposure to POPs. It is of substantial interest to identify sources of variability in the measured human concentrations. Biomonitoring studies often obtain additional information on the sampled individuals through questionnaires. Collected data generally include
age at sampling, date of sampling, gender, body mass index, number of children and age at birth, breastfeeding habits, and detailed dietary composition. While such data can be used to find statistical associations between measured contaminant exposures and potential influential factors, statistical analyses cannot identify causal relationships and provide ultimately no mechanistic understanding of the processes underlying human POP exposure. However, the same questionnaire responses can also be used to individualize the parameterization of the human in mechanistic exposure models. In other words, instead of only calculating average population exposure, the models can be used to simulate the distinct exposure histories of all sampled human participants individually. Here we present the results of three studies simulating the exposure of individuals to polychlorinated biphenyls (PCBs) using the environmental fate and bioaccumulation model CoZMoMan or similar mechanistic approaches. The models combined data from multiple sources including measured and estimated population average exposure. Also the range of predicted PCB exposures generally agrees with the range of measured exposures. Trends with key variables, such as BMI and age, were mostly similar for the measured and simulated concentration data. However, in two studies the models attributed more of the variability in PCB exposure to inter-individual differences in dietary composition than a statistical analysis of the measured data would support. The measured data indicated that there were other sources of variability not currently accounted for in the models.

678 Cleaning Product Ingredient Safety Initiative: Product-Type-Specific Exposure Assessment

S. Williams, Baylor University; M.C. Ciarlo, EA Engineering, Science, and Technology; C. Horne, EA Engineering, Science, and Technology / Water and Natural Resources; B. Gregg, Soleil Consulting; P.C. DeLeo, American Cleaning Institute; B.W. Brooks, Baylor University / Dept of Environmental Science

The Cleaning Product Ingredient Safety Initiative (CPISI) is a multi-year effort to conduct a screening-level risk assessment for all identified chemical ingredients in the consumer cleaning products manufactured by the member companies of the American Cleaning Institute (ACI). One objective of the CPISI was to calculate deterministic estimates of intake for each cleaning product through each possible exposure route. The base requirements for the estimates are 1) the concentration at the point of exposure, 2) the frequency and duration of product use, 3) the amount used, 4) the body weight of the exposed individual, and 5) route-specific parameters (respiratory fraction, skin contact area, etc.). In this exercise, only intended uses of the products were considered. To establish concentrations of ingredients in the products, we revisited data gathered earlier in the program, and also gathered formulation information (i.e., frame formulas) for each product type from trade literature, company surveys, and other resources. Ranges of concentrations for each ingredient in each product within the scope of the CPISI were gathered, in the context of its functional use class (FC). Other exposure parameters were collected from previous efforts on habits and practices conducted by the American Cleaning Institute, and from additional resources such as EPA’s Exposure Factors Handbook and the Human and Environmental Risk Assessment for Cleaning Products. Estimates of exposure were calculated and incorporated into a database, and when ingredients were listed in multiple product types, estimates of aggregate exposure were also developed and integrated. This effort has culminated in development of a database which links cleaning products to their respective types to ingredients and ingredient functional use classes (FC) to relevant exposure models and ultimately to an upper-bound point estimate of intake under typical conditions of product use. This database will be available through ACI’s Science portal. Future efforts will consider the transitions to safer ingredients for each ingredient. Should the margin of exposure exceed an acceptable threshold, refinement of the exposure estimates may become necessary. Numerous opportunities exist for refinement of these exposure estimates, including re-examination of ingredient concentrations and use patterns for each product.

Effect Modelling of environmental systems - extrapolation and prediction of species response (II)

679 Benefits of ecological modelling for risk assessment

M. van Dongen, J. Genau, Grun, D. Schrap, Modelling

While population modelling and other mechanistic modelling approaches are used occasionally as a higher tier risk assessments of chemicals, these methods are not yet fully integrated into the present risk assessment scheme. This is partly due to the fact that population models are complex and not easily to understand by non-experts, but it is also due to concerns regarding the reliability and realism of population models. These concerns are intensified by uncertainty in complex population models and that some data needed to address a specific risk may not be available. We therefore evaluate the uncertainties and the realism involved in standard risk assessments and show how many of them can be addressed using modelling. We show which benefits models offer to risk assessors, particularly for reducing uncertainties included in standard risk assessments. While all data used in standard risk assessments also includes additional data at higher level of resolution, much more additionally are used currently considered in the latter. In addition, effects can be studied in greater depth, determining which ecological or toxicological factors mainly influence the risk for a particular species and under which conditions/scenarios a risk may occur. Safety factors are used in standard risk assessment, which may or may not be large enough to cover uncertainties and natural variability. However, modelling approaches are often fully probabilistic, ensuring that even rare events (e.g. particularly high exposure in few animals or effects in years with very unfavorable weather conditions) can be studied. The comparison of population-level risk assessments vs. standard risk assessments show that the risk assessment has greatly benefited from the possibility to reduce uncertainties of standard risk assessments by taking all our knowledge of a species into account instead of focusing only on exposure and toxicity.

680 Modelling effects of variable herbicide exposure to terrestrial plant communities in field boundaries

J. Reeg; T. Schad, EnSAG; K. Körner, University of Potsdam; A. Solga, Ecology; T.G. Preuss, Bayer CropScience / Environmental Modelling; F. Jeltsch, University of Potsdam

Plant communities of field boundaries are potentially impacted by herbicide exposure due to drift. The intensity of exposures varies e.g. due to wind and application. Risk assessments aim to evaluate the impact on plant communities. With respect to ecotoxicological testing, guidelines (OECD, US EPA) define experiments on individual species. They do not take interactions of individuals within or amongst species into account. Especially these intra- and interspecific interactions influence plant community dynamics. By linking a landscape-scale exposure model (XPlicit) to a mechanistic plant community model (IBC-grass), we simulate the effect of variable herbicide exposure on herbaceous plant communities neighbouring treated fields. IBC-grass is an individual-based plant community model for grasslands using the trait-based plant functional type (PFT) approach to cover a wide range of species characteristics. Based on an extensive literature research, we compiled a list of herbaceous plant species typically forming field boundary communities in Europe. According to that, IBC-grass was parameterized and the regional species pool classified into PFTs. Due to the individual-based approach of IBC-grass, we were able to transfer individual-level risk effects (as a species effect distribution; XPlicit) to PFT individuals within IBC-grass. Exposure effects were simulated on local patches of 3 m² along a distance gradient from the crop field. Species effect distributions were generated by XPlicit. On community level, effects on functional diversity and richness as well as on biomass were analyzed. On population level within the community context, effects on population size, biomass and the recovery potential were studied. Close to the field edge, simulated PFTs showed - besides short-term direct effects - also long-term indirect effects in the community context that are related to interactions between individuals. Variable exposure showed that years of low herbicidal disturbance have a stronger effect on reducing long-term risk as opposed to direct effects. Applying our model approach makes it possible to upscale from individual herbicide effects to community responses. Including variable herbicide exposure adds more realism to our mechanistic model approach at landscape-scales. Thereby, potential future assessment endpoints can be addressed.

681 Coupling aquatic IBMs and ecosystem models to interoperate effects from the laboratory to the field

T. Strauss, Research Institute gaiac / Research Institute Gaiaec; F. G asi, Rifen GmbH; M. Hammers-Wirtz, Research Institute gaiac; P. Thorbek, Syngenta / Environmental Safety; T.G. Preuss, Bayer CropScience / Environmental Modelling

For the application of mechanistic population models in ecological risk assessment, an important question is still under debate: Which level of complexity is needed for a realistic and accurate description of population dynamics? In this study, we combine a laboratory individual-based population model (IBM) for D. magna (IDamiP) and a complex stoichiometric lake model (StoLaM), to create the DaLaM (Daphnia Lake Model). We used DaLaM to predict population dynamics of two ecotoxicologically relevant laboratory test organisms, D. magna and the green alga Desmodesmus subspicatus, under realistic field conditions. In DaLaM, the variable environmental conditions such as light, temperature, and nutrient cycling are realistically simulated. Also for the population dynamics of both planktonic species, we integrate a high number of biological processes, but we deliberately and drastically simplify the interorganism complexity including only two trophic relations for two species. For model testing, we used data from four sets of laboratory and mesocosm studies lasting several months, and from a 2-year mesocosm study including two winter seasons. Our aim is to demonstrate the predictive power of detailed models in which the parameterization of the different biological processes was based on laboratory experiments, for extrapolating from individuals in the laboratory to populations in the field. We will show DaLaM’s ability to predict the overall population pattern of phytoplankton and daphnids in the mesocosms, indicating that the essential mechanisms and main drivers of the system have been included in the present modeling approach. Also the possibility to simulate direct and indirect effects on the Daphnia-phytoplankton interactions for scenarios using insecticides as well as herbicides, will be discussed.

682 A new modeling approach for population level risk assessment of lagomorphs

J. Kleinmann, WSC Scientific GmbH; M. Wang, WSC Scientific GmbH / Dept Efate Modelling
Addressing data collection and computational challenges in LCI (II)

684 The importance of going beyond standard LCI databases: Lessons from a meta-analysis of potable water supply system LCAs

N. Meros, The Porter School of Environmental Studies, G. Thoma, University of Athens / School of Environmental Design Engineering; N. Chontos, Tel-Aviv University / The Recanati Graduate School of Business Administration; Y. Garb, Ben-Gurion University of the Negev / Blaustein Institutes for Desert Studies Social Studies Unit

The life cycle assessments (LCAs) for an overwhelming portion of products and services must draw on estimates of the environmental impacts of potable water. Currently to a hundred LCAs have been published characterizing the impacts of water supply systems, however, the great diversity of the systems and their location yield a diversity of LCA results. Thus, in the absence of localized Life Cycle Inventories (LCIs), most analyses draw their impact figures from a standard life cycle inventories, whose applicability to a given local case is uncertain. This has led for a call for increased harmonization of LCA efforts to allow comparison and pooling of results. Here we describe a comprehensive and rigorous review of LCAs of water-supply systems and sub-systems, which allows us to better understand the sources of variation in these studies, and identify those elements of the water-supply system with the consistently highest environmental impacts. This review utilized a systematic differentiation of the sub-systems, functional units, and system boundaries referenced in each of over 100 candidate studies to produce a comparable subset of 34 LCA studies. Next, statistical techniques (cluster analysis and analysis of means) were used to isolate and validate the main causes of variation and the sub-systems in which these are most pronounced. It shows the presence or absence of desalination (and thermal desalination) as the main driving determinant of the environmental impacts of water supply systems, with other factors in a secondary role. Our results show the importance of LCA that goes beyond standard LCI databases to consider the local context of water production, and points the way to doing so without conducting a localized analysis in each case. Given the large variation we describe in the impacts of water supply systems (from 0.2 to 3.4 kg CO2-eq/m3 of supplied water), a LCA of water-intensive products drawing from a standard LCI databases could be inaccurate in many settings, especially in a region with desalination—an uncertainty that can be reduced considerably by taking this into account.

685 Temporally disaggregated data for the life cycle assessment of services provided by ICT

T. Dondres, CIRAIG; E. Maurice, CIRAIG - École Polytechnique de Montréal / Chemistry; K. Nguyen, Synchromedia École de Technologie Supérieure; Y. Lemeux, Ericsson Canada; M. Cheriet, Synchromedia École de Technologie Supérieure; R. Samson, École Polytechnique de Montréal / Department of Chemical Engineering

The dynamic functioning of the modern society relies increasingly on information and communication technologies (ICT). However, despite that ICT have some positive effects on the society, they also have impacts on the environment. Indeed, it was estimated in 2007 that ICT contributed to 2% of the global anthropogenic greenhouse gas (GHG) emissions, the same amount that the aviation sector. Therefore, many efforts are being made to lower the overall environmental footprint of ICT. The life cycle assessment (LCA) could be a good candidate for that purpose, but it faces several methodological challenges. In this study, the focus is made on electricity modeling in the LCA of ICT. Indeed, both the electricity generation by power plants and the electricity demand by ICT usually vary all the time independently. However, these temporal phenomena are commonly neglected in LCAs. To improve the accuracy of the dynamic LCA approach, the temporally disaggregated data, has been used to model environmental impacts attributed to the electricity consumption of data centers in different regions of Canada. Additionally, a dynamic consequential LCA approach has been developed to minimize in real time, the GHG emissions of two data centers working in cloud computing. Results show that an LCA based on annual aggregated data would underestimate by 10% the GHG emissions of the data centers during the use phase when compared to the dynamic LCA using real-time data. Regarding the real-time GHG emissions minimization of the cloud computing: the dynamic consequential LCA allows for a reduction of 35% of the cloud computing GHG emissions when compared to the conventional LCA. The results obtained in this project highlight that temporal aspects should not be neglected when studying dynamic LCA. With the emergence of ICT, it becomes particularly important to consider the regional and temporal aspects of both generation and consumption of electricity in LCA and carbon footprint assessment. Moreover, the case of systems optimized in real-time thanks to ICT is problematic since the current methodologies are not able to compute the reduction of GHG emissions achieved by the smart management of electricity in such systems. Therefore, there is a need to update the current LCA and carbon footprint guidelines for the ICT sector. It is recommended to use regional temporally disaggregated data to evaluate ICT when ICT power demand and regional electricity generation vary over time.

686 Indoor air quality impacts in a regional energy conservation district: Life cycle model and baseline data collection

M.M. Bilec, W.O. Collinge, University of Pittsburgh / Civil and Environmental Engineering

The potential of green buildings to significantly improve occupant health and productivity while mitigating the effects of anthropogenic climate change and reduced energy use remains unfulfilled. However, recent reports call for a shift to set aggressive energy and greenhouse gas emission reduction strategies. We have introduced the term Energy Conservation Districts (ECDs) to describe such initiatives which are taking place in a specific geographic area on the order of a city neighborhood or business district. In this research we are conducting an indoor air quality (IAQ) pilot study to establish baseline IAQ conditions and associated human health and productivity impacts in an ECD located in Pittsburgh, PA, USA. Factors influencing energy use in buildings, such as ventilation and envelope modifications, can influence IAQ directly, while indirect effects may be linked to geographically co-located energy supply and transport processes. We outline a geographically specific LCA model coupling building energy use models with both...
direct and indirect life cycle impacts, showing how proposed energy savings measures could influence IAQ. The Use-Tox life cycle impact assessment (LCIA) model is used as a source of effect factors, while exposure assessment is assessed with a modified indoor-outdoor compartment model compatible with Use-Tox. We also outline a protocol for data collection to establish baseline IAQ conditions within the subject ECD. The baseline data will be used to generate an existing conditions LCIA model which can be adapted to simulate proposed energy use reduction measures.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (II)

Phase two of inter-laboratory validation of the Xenopus Embryonic Thyroid Signalling Assay

S. Mothre, A. Sebillot, A.J. Tindall, Watchfrog S.A.; T. Iguni, National Institute for Basic Biology / Molecular Environmental Endocrinology; K. W. Buchholz, Biological Sciences, Allen Hamilton Inn / Military Health System Environmental; D. Demenex, MNHN/CNRS; G.F. Lemkine, D. Paquet, Watchfrog S.A.

The Xenopus Embryonic Thyroid signalling Assay (XETA) was designed as a screening assay to provide information on the potential of a test substance to alter the normal functions of the thyroid system. The XETA provides a rapid (<72h) way to measure the response of embryonic stage tadpoles to potential thyroid disrupting chemicals, allowing a efficient method for screening thyroid disruptors. In addition to serving as a quick screen for thyroid active chemicals, XETA, could serve as a potential alternative method to the in vivo Amphibian Metamorphosis Assay (AMA - OECD TG231). The AMA test is based on the study of the metamorphosis of tadpoles after three weeks of exposure to a given chemical, and includes histological examination of the thyroid gland. XETA could provide an alternative approach for be performed quickly, providing information that would be useful for screening large number of molecules or testing environmental samples that couldn’t be stored or sampled in large quantities. The objective of the validation study is to establish the relevance of the assay by assessing its sensitivity to detect disruption of the thyroid system by compounds active at different points within the thyroid system. The validation is intended to determine the performance and transferability of the assay across a range of both experienced and naïve laboratories. These techniques often need the use of other software than the ones commonly used in LCA (e.g. Python language here). Further research regarding the case study will concern a better understanding of the model. However, these techniques often require an evaluation of how to deal with stocks, and the uncertainty/sensitivity analyses related to data collection (uncertainty characterization). To better understand the potential applications of the method these techniques often need the use of other software than the ones commonly used in LCA (e.g. Python language here). Further research regarding the case study will concern a better understanding of the model. However, these techniques often require a better specification and GSA showed low derivatives. Global sensitivity analysis (GSA) explores the entire space of the inputs and calculates their influence and interactions (non-linear effects (method of elementary effects or Fourier/Sobol variance decomposition). Noise characterization models were used to evaluate the potential benefits of a new car tire as compared to its base version and tested through uncertainty analysis (MC). A few LCA studies explored the use of fuzzy intervals which require less information. Hybrid method samples both on probability and possibility distributions. Finally, analytical methods calculate the variance of the inventory matrix based on Taylor series expansion. Local sensitivity analysis (LSA) studies the effects of small inputs variations on the model output, by varying one parameter at the time (OAT), building scenarios or calculating derivatives. Global sensitivity analysis (GSA) explores the entire space of the inputs and calculates their influence and interactions (non-linear effects (method of elementary effects or Fourier/Sobol variance decomposition). Noise characterization models were used to evaluate the potential benefits of a new car tire as compared to its base version and tested through uncertainty analysis (MC), LSA (OAT) and GSA (Sobol), Due to lack of data, several distributions for a key parameter were tested and led to different conclusions, which highlights the need of refinement and numerical experimentation. As a result, the statistical methods, LSA and GSA identified the same parameters which needed a better specification and GSA showed low influence of interaction effects. Statistical methods require a good knowledge on the input uncertainties, which was an obstacle for the tire noise results interpretation. Some approaches allowed characterizing uncertainty with little information and GSA quantifies the influence of parameters and the interaction effects. As a result, it is better to use the results of the LSA in combination. The statistical methods are implemented in the Python language.

Endocrine Disrupting Potential of Tebuconazole: a Xenopus laevis 27-day study

R. Paulsen, University of Copenhagen / Department of Pharmacy; X. Luong, University of California Berkeley / Department of Integrative Biology; M. Hansen, University of California, Berkeley / Civil Environmental Engineering Integrative Biology; B. Styrishave, Dep. of Pharmaceutics & Analytical Chemistry; T. Hayes, UC Berkeley / Integrative Biology.

Tebuconazole, a class of agrochemicals, which have shown high capacity for causing endocrine disruption. In this group we find tebuconazole, a class of agrochemicals, which have shown high capacity for causing endocrine disruption. In this group we find tebuconazole, which is a member of the triazole family. Tebuconazole is known to affect the thyroid system by compounds active at different points within the thyroid system. The thyroid system is responsible for the production and release of thyroid hormones, which regulate various physiological functions. Exposure to tebuconazole may disrupt the normal thyroid function, leading to various health effects. The main mechanism of action for tebuconazole is disruption of the enzymes CYP17 and CYP19 in the thyroid gland. This disruption can lead to the production of abnormal thyroid hormones, which can disrupt the normal thyroid function and lead to various health effects.

Tebuconazole is used extensively in agriculture as an active ingredient in various formulations to control diseases in crops and vegetables. The use of tebuconazole in agriculture has raised concerns about its potential effects on human and environmental health. Tebuconazole can be found in various environmental matrices such as soil, water, and air. The presence of tebuconazole in these matrices can pose risks to human health and ecological systems. Tebuconazole is known to be persistent and bioaccumulative in the environment, leading to long-term exposure in various compartments.

To address these concerns, studies have been conducted to evaluate the potential endocrine-disrupting effects of tebuconazole. These studies often involve in vitro and in vivo assays to assess the potential endocrine-disrupting effects of tebuconazole. In vitro assays are used to evaluate the effects of the compound on specific target tissues or cells, while in vivo assays are used to evaluate the effects on whole animals or organisms.

However, these assays often require substantial resources and expertise. To address this challenge, a new screening assay, XETA, was developed. XETA is a high-throughput, high-content assay designed to evaluate the potential endocrine-disrupting effects of compounds using Xenopus laevis tadpoles. XETA is based on the observation that exposure of Xenopus laevis tadpoles to endocrine-disrupting compounds leads to morphological and physiological changes.

The assay consists of a panel of endocrine markers, which are sensitive to the exposure to endocrine-disrupting compounds. The assay measures the activity of these markers in response to the exposure, providing a quantitative measure of the endocrine-disrupting potential of the compound.

The assay is highly sensitive and specific, allowing the detection of low concentrations of endocrine-disrupting compounds. The assay also provides valuable information on the mechanisms of action of endocrine-disrupting compounds, which can help to identify potential targets for further research.

In conclusion, XETA is a valuable tool for the screening of endocrine-disrupting compounds in agriculture. It provides a high-throughput, high-content assay for the detection of endocrine-disrupting effects, which can help to identify potential targets for further research and development of safer and more environmentally friendly agrochemicals.
691 Lidar affinity of the Lymnaea stagnalis estrogen and retinoid-X receptors (LsER and LsRXR): implications for detecting endocrine disruptors
A. Boulatourd, IRCM Iserin U896 - Université Montpellier1; M. Grimaldi, IRCM, INRA, UMR Emotion et Apprisson, INSERM, U896; M. Coutellec, INRA / Ecology and Ecosystem Health UMR INRA Agrocampus Ouest; A. Benaouda, INRA / UMR LESI 905; Y. Echassier, INRA; W. Bourguet, CBS CNRS UMR5048 - INSERM U1054; P. Balague, INSERM U896 IRCM; J.L. Legaëd, Bayer CropScience / Environmental Safety

The freshwater gastropod *Lymnaea stagnalis* has been selected as a test species for the development of an OECD Guideline for proreotoxicity testing in molluscs. This *in vivo* test will appear as a Mollusc Partial Life-Cycle Assay at Level 4 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (EDs). However, in *L. stagnalis* as in other mollusc species, the existence, properties and ligand-affinity to ED-binding receptors are poorly documented and even controversial, so that the endocrine origin of adverse effects on individuals' reproduction cannot be ascertained. The present study was therefore designed to characterize the properties and ligand-affinity of ED-binding receptors of *L. stagnalis*. The genomic resources recently acquired in *L. stagnalis* and available for other molluscs were used to sequence LsER and LsRXR genes. Expression vectors of these genes were synthesized and used for transient cell transfection. Transactivation and binding tests were then performed with ligands known to bind human ER and RXR (hERs and hRXRs). In parallel, the ligand binding domains of LsER and LsRXR were modelled to characterize molecular interactions between LsER or LsRXR and the tested ligands. The results showed that LsER activity is constitutive and not mediated by known ligands of hERs, including estradiol, its natural ligand, and bisphenol A. In contrast, LsRXR can be activated by its natural ligand 9-cis-retinoic acid, and also by pharmaceutical (e.g., CDX254) and environmental (e.g., organotins) ligands. This *in vitro* approach will contribute to fill gaps on EDs on molluscs and support interpretation of mechanisms underlying adverse reproductive effects of chemicals in *L. stagnalis* in the context of the forthcoming OECD Guideline.

692 Linking high throughput screening data of chemicals and environmental models to endocrine activity and sex organ deformities in experimental animals

We have a long standing history in developing and applying robust CALUX® receptor active gene array as hazard identification tool (food-, water-, -environmental and epidemiological monitoring). In the EU FP7 project ChemScreen, we focused on the use of these models and complementary technologies to set up a broad high throughput screening system for endocrine disrupters and reproductive toxicants. The assays we used were highly selective for specific molecular pathways. This allowed more straightforward validation of the panel and linkage of its results via so-called adverse outcome pathways (AOPs) to test results in experimental animals. Using a mechanistic approach a high throughput panel of assays has been developed which can be used to predict endocrine activity of single chemicals and environmental mixtures. We showed that this mechanistic approach can also be used to validate assays and link them to adverse outcome pathways and that adverse effects on experimental animals can be identified by a closer link to current guideline tests can be made. Additional steps towards regulatory acceptance include validation and establishment of trigger values.

693 Major contribution of bisphenol A to estrogenic activity in landfill leachate as revealed by effect-directed analysis
L. Fuster, LPTC-EPOC, University of Bordeaux vs UMR EPOC LPTC; L. MAZEAUS, Irstea, Unité Hydrostyesmètes et bioprocédés; K. LE MENACH, LPTC; A. GUEINE, Irstea Antony / Unité Hydrostyesmètes et bioprocédés; M. DÉVIER, LPTC-EPOC, University of Bordeaux / Oceanic and Continental Environments and Paleoenvironments; N. CREUSS, INERIS; L. ROULLAC, Irstea Antony / Unité Hydrostyesmètes et bioprocédés; P. MAZELLIER, ANSES / Member of the Working Group; S. AIT-Aissa, INERIS / Ecotoxicology Unit; H. Budzinski, University of Bordeaux vs UMR EPOC Epitech

Landfill leachates are contaminated by a wide diversity of organic compounds and especially by endocrine disrupting chemicals. These complex mixtures can be leached into soils and groundwaters by advective transfer through holes or diffusion through geosynthetic liners and constitutes a risk for aquatic ecosystems. Because of complexity and variability of these matrices and the restricted number of molecules focused in targeted chemical analyses, selection of relevant molecules for environmental monitoring is often laborious. Effect-directed analysis combining fractionation step, with chemical and biological analyses might allow to identify some compounds responsible for activities of observed effects. Investigations were conducted on an untreated leachate of municipal waste disposal. After a first fractionation step by RP-HPLC, estrogenicity of crude extract and 40 fractions was assessed by in vitro bioassay on the reporter cell line MELN. An estrogenic activity (260 ng E2QOL) was detected in raw extract of landfill leachate. In order to bring out estrogenic molecules not targeted by chemical analysis in the global extract, this extract was fractionated on cellulose, followed by chemical analysis. In this work, allows to identify bisphenol A as main contributor to estrogenic activity in this landfill leachate and brings to light the presence of polar estrogenic compounds. This method can be valuable to prioritize hazardous molecules in landfill leachates.

694 Altered reproductive capacity of Japanese medaka exposed to maternally transferred TBCO
S.B. Wiseman, University of Saskatchewan / Toxicology Centre; J. SUN, Toxicology Centre; S. Tang, School of Environment and Sustainability; D.M. Saunders, University of Saskatchewan / Toxicology; h. peng, Toxicology Centre; M. Hecker, University of Saskatchewan / School of the Environment and Sustainable Toxicology Centre; J.P. Giery, University of Saskatchewan / Toxicology Center

Great efforts are being made to assess risks to exposure to endocrine disrupting chemicals (EDCs), a group of chemicals that can impair reproduction resulting in declines in populations of fishes. The brominated flame retardant (BFR), 1, 2, 5, 6-tetabromocyclooctane (TBCO), is an EDC. Although most studies focus on effects of TBCO on sexually mature fishes, early-life stage fishes, including embryos, respond to EDCs. However, little is known about long-term consequences of exposure that occur only during embryonic stages of development. Therefore, studies were conducted to determine effects of TBCO on 2 generations of Japanese medaka to investigate whether exposure only to maternally transferred TBCO affects reproductive capacity of adults. Fecundity of the F0 generation exposed to TBCO through their diet was lesser by 18% compared to controls. Cumulative fecundity of F1 adults given the TBCO through their diet but raised from embryos of F0 adults given the control diet was lesser by 30% compared to fecundity of F1 adults of the same lineage but given the control diet. However, fecundity of F1/2 adults raised from embryos of F0 adults given the diet of TBCO was only lesser by 8% compared to controls. Fertilization of eggs was not quantified in the F2 generation. However, fertilization of eggs by F1 generation exposed to TBCO via maternal transfer and that were given the diet of TBCO was lesser by 18% compared to fertilization of eggs by F1 generation that were not exposed to TBCO via maternal transfer but were given the diet of TBCO. This suggests that exposure only to maternally deposited TBCO differentially effects reproductive performance of male and female fish. Mechanisms of effects are not known. Molecular responses of embryos to TBCO might confer greater tolerance to TBCO during reproduction. Results of transcriptomic and proteomic studies to elucidate effects of TBCO on embryos will be discussed. To begin to understand if there is an epigenetic basis to these effects the reproductive capacity of adults from embryos exposed to waterborne TBCO will be presented. Overall, results suggest that responses of embryos to maternally transferred TBCO are an important determinant of reproductive capacity of sexually mature fishes.

Carbonaceous materials for contaminants remediation

695 The sorption strength of neutral organic compounds to biochars: a novel remediation technique
S.E. Håle, Norwegian Geotechnical Institute / Environmental Engineering; H.H. Lip, NGI Environmental Engineering; D. Kupryianchyk, Norwegian Geotechnical Inst.; G. Cornelissen, NGI

The sorption strength of neutral organic compounds to biochars was reviewed and subsequently related to biochar and compound properties. From 29 studies, 509 chemical analysis in the global extract, this extract was fractionated on cellulose, followed by chemical analysis. In this work, allows to identify bisphenol A as main contributor to estrogenic activity in this landfill leachate and brings to light the presence of polar estrogenic compounds. This method can be valuable to prioritize hazardous molecules in landfill leachates.
The sorption strength of organic compounds to biochars increased with increasing biochar production temperature, biochar SA and organic pollutant hydrophobicity and decreased with biochar O/C ratio and biochar H/C ratio. A combination of these variables resulted in the following multiple parameter linear regression: log $K_{OC} = (0.15±0.06) \log K_{OC} + (3.7±0.74) \log T + (0.75±0.15) \log SA + (1.74±0.28) \log OC$ $+(0.49±0.19) \log HC + (-6.53±1.67) r^2 = 0.62$, root mean squared error $= 0.94$, $n = 15$. To confirm this hypothesis, initial screening to identify biochars that are optimal for use as pollutant sequestering agents for the purpose of reducing the risk of organic pollutants in agricultural areas or the environment. Biochars produced at high temperatures are preferable as sequestering agents, with biochars produced at temperatures greater than 400 °C sorbing the most consistently. The choice of feedstock has a smaller effect on organic sorption affinity and thus the selection of a biomass material should primarily be based on logistics, such as abundance as a waste product and low transport costs.

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**ADSORPTION OF POLAR AND IONIC COMPOUNDS TO PYROGENIC CARBONACEOUS MATERIALS**

L.J. Pignatello, Connecticut Agricultural Experiment Sta. / Environmental Sciences; F. Xiao, C. Lattao, The Connecticut Agricultural Experiment Station / Environmental Sciences

This talk will describe recent work on adsorption to pyrogenic carbonaceous materials (PCMs). It will emphasize polar and charged compounds, which have received much less attention than hydrophobic compounds, but have a colorful chemostry at PCM interfaces. It will go beyond traditional PCM to include ionic interactions, pi-pi interactions, strong hydrogen bonding, competitive effects, organic matter fouling, and critical structural characteristics of the PCMs that govern sorption. The talk will also reveal one way in which modification of biochars can enhance sorption.

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**The power of power: Electrokinetic control of PAH interactions with exfoliated graphite**

L.Y. Wick, Helmholtz Centre for Environmental Research UFZ / Environmental Microbiology; J. Qin, Helmholtz Center for Environmental Research UFZ / Environmental Microbiology; A.M. Moustafa, University of Alberta / Civil and Environmental Engineering; H. Harms, Helmholtz Centre for Environmental Research UFZ; M. Gamal El-Din, University of Alberta / Department of Cival and Environmental Engineering

Polycyclic aromatic hydrocarbons (PAH) are commonly found in natural and oil field produced waters. Without adequate treatment PAH contaminate the ecosystem and cause deleterious impacts on human health. Various treatment approaches for process water have been developed including chemical modifications to adsorbents. Among them, exfoliated graphite (EG) has been attracting particular interest. Having a low density, a high surface area and wide pore size distribution of 2 nm to 10 µm, EG exhibits an exceptional sorption capacity for petroleum and its constituents. Due to its comparably high cost, however, novel approaches hybridizing sorption with physical and biological treatment approaches are needed. Here we use EG as a carbonaceous model sorbent in order to assess the effect of weak direct current (DC) electric fields on the EG sorption capacity for the PAH phenanthrene. When an electric field is applied to a matrix subserved in an ionic solution, it invokes electromigration, electrophoresis, and electroosmosis (EOF). EOF is the surface-induced movement of pore fluids in an electric field, usually from the anode toward the cathode. EOF is more efficient in fine-grained (with meso- and micropores) than in macroporous materials. The conductivity of fine-grained matrices for pressure-driven (e.g. hydraulic) liquid flow is extremely small and leads to quasi stagnant water in micro- and mesopores. Molecular diffusion hence is limiting both access of sorbates to and release of sorbates from such pores as potential sites of high sorptive capacity for sorbates. Hence, EG-filled micro-reactors were exposed to a weak DC in presence of PHE to challenge the hypothesis that DC-fields may lead to increased EOF-mediated PHE transport to poorly accessible EG sorption sites and to increased sorption rates and/or decreased desorption rates of as compared to DC-free controls. Our data confirm this hypothesis. They show that EOF leads to increased PHE entrainment in EG pores and both increased sorption rates and >99% reduced desorption rates in presence of DC. Such ‘power of power’ may allow for tailor-made regulation of the sorption/desorption kinetics of chemicals to/from carbonaceous sorbents e.g. in water treatment or in biotechnology to control the bioavailability of substrates to transforming microorganisms. Current studies using activated charcoal support our findings of EG.

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**Effects of activated carbon amendment on benthic organisms Lumbricillus variegatus and Chironomus riparius in PCB contaminated sediments in freshly amended and aged systems**

L. Nybom, Department of Biology; S. Abel, University of Eastern Finland / Department of Biology; G.C. Waissi-Leinonen, University of Eastern Finland; K. Maenpaa, University of Eastern Finland / Department of Biology; M.T. Leppanen, Finnish Environment Institute / Laboratory Centre; J.V. Kukkonen, University of Jyvaskyla / Biological and Environmental Science; J. Akkanen, University of Eastern Finland / Department of Biology

Activated carbon (AC) amendments have been studied as a new stabilizing method for contaminated sediments. The sorption efficiency of AC toward hydrophobic organic compounds (HOCs) has been shown in several studies. Recently the focus has been turned also to the possible secondary effects of AC amendments. The result relating to secondary effects has been somewhat contradictory and especially sediment dwelling organisms has been shown to be sensitive to AC amendments. The aim of this study was to investigate the efficiency of AC to reduce bioaccumulating concentrations of PCBs and possible adverse effects on benthic organisms, main focus being on how long contact time affected the effects of AC amendments. Lumbricillus variegatus and Chironomus riparius were used as test organisms. Coal based AC (ø 63-200 µm) was mixed in natural sediments from PCB contaminated areas and let to stabilize for two weeks or three years after amendment. AC amendments proved to be efficient in reducing PCB bioaccumulation and the sorption capacity remained strong even after three years of AC-sediment contact time. However, adverse effects were observed on L. variegatus biomass and egestion both with two weeks and three years AC-sediment contact times. Reduction in C. riparius growth was observed in addition with indications of AC amendment related delay in emergence especially in the second generation midges in the experiments were AC-sediment contact time was two weeks. On the other hand low dose of AC (0.5% sediment dry weight) increased production of eggs in some of the studied sediments. Responses observed within this study emphasise the importance of site specific characterization when remediation measures are designed, by knowing the local conditions the applied doses may be adjusted to avoid secondary effects as far as possible. Sediment dwelling organisms may be sensitive to carbon amendment due to the exposure both internally and externally and thus they may also be suitable indicator species for the secondary effects of AC.

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**PCB reduction in fish after AC amendment to sediment**

U. Ghosh, University of Maryland Baltimore County / Chemical Biochemical and Environmental Engineering; H. Fadaei Khoei, University of Maryland Baltimore County / Civil and Environmental Engineering; E.R. Patmon, University of Maryland Baltimore County / Chemical Biochemical and Environmental Engineering

While much work has been done demonstrating the effect of strong sorption of PCBs in sediments on bioaccumulation in benthic invertebrates, exposure to fish is less well understood. Black carbon in sediments present natively, or added as an amendment can greatly reduce porewater concentrations of PCBs, resulting in reduced flux from the sediment to overlying water and reduced bioaccumulation in benthic organisms. In the present study we performed laboratory aquaria experiments, modeled, and a full-scale remediation in a small lake, to explore how PCB sorption in sediments impacted exposure pathways and bioaccumulation in fish. Impacted sediments from a contaminated river treated with activated carbon to reduce bioavailability was used in the laboratory experiments along with untreated and control sediments. Laboratory results showed that porewater PCB concentration in impacted sediment was reduced by two orders of magnitude upon AC amendment with 4.5% powdered activated carbon. In the full-scale remediation, reduced the PCB uptake in fish by 87% after 90 days. Freely dissolved concentrations in porewater and overlying water was measured by passive sampling and incorporated in equilibrium and kinetic bioaccumulation models for predicting uptake by fish. The fish exposed to untreated sediment did not reach equilibrium after 90 days of exposure in the laboratory. Uptake prediction with the kinetic model was generally within a factor of 2 compared to observed values for dominant PCB congeners. Our results indicate that by tracking changes in freely dissolved porewater and overlying water PCB concentrations, it is possible to predict effectiveness of sediment remediation in reducing PCB uptake in the food chain, including fish. The latest results from a full-scale field implementation of AC amendment to a 5-acre lake and food web monitoring will also be presented.

**Sustainability of Mediterranean olive oil production**

**700**

**Review of Good Practice in Olive Oil Production: Integrating Ecosystem Services and Sustainability Thinking**

S.E. Apitz, SEA Environmental Decisions Ltd

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**Life Cycle Assessment in the agri-food sector: olive oil**

B. Notarnicola, Università degli Studi di Life Cycle Assessment in the agri-food sector

**702**

**Sustainable olive oil production by effective utilization of olive mill wastes**

A.G. Vlyssides, National Technical University of Athens; N. Van der Smissen, National Technical University of Athens / School of Chemical Engineering

All olives’ treatment processes (olives, olive oil, olive wooden residue) produce liquid and solid wastes, which are considered toxic. The treatment of these wastes
by using conventional technologies (aerobic/anaerobic biological treatment, incineration, gasification etc) proved to be neither technically nor cost effective. This fact treats the olive oil production by complete cease due to the serious environmental problems caused. The proposed technology includes the detoxification of wastewater by Fenton oxidation reactions and following their utilization by biological treatment methods. The latter is achieved by the implementation of anaerobic digestion process on the oxidized wastewater and the consequent methane production and by co-composting the oxidized or/and anaerobic digested wastewater with solid wastes (olive mill wooden residue, leaves, branches etc.), leading to the production of a high quality soil conditioner. The biogas produced could be utilized for in situ thermal and electrical energy production. The two biological processes used (anaerobic digestion and composting) are ideal combined, since the anaerobic digested wastewaters are totally used in composting process, while the excess thermal energy produced by biogas utilization can accelerate the aerobic biological processes resulting in a high quality biological fertilizer. The proposed technology is effective and simple to be implemented. Wastewaters derive either from two or three phase olive mill could be treated. This technology can be implemented either in one olive mill scale or in central wastewater treatment plants. The proposed technology can not only effectively face the serious environmental problems caused by olive mill wastewater disposal, but it poses new perspectives in olive trees cultivation and olive oil and its by-products market. The soil conditioner is produced exclusively from plants, and it has been proved to be ideal for the biological cultivation of many products such as citrus, vineyards, aromatic herbals, asparagus etc. Thus, it can make the biological olive trees cultivation effective, because the use of chemical fertilizers is totally eliminated and the economical profit perspective is promising, since the price of biological olive oil is 1.5 times higher than the respective price of the olive oil deriving from “chemical” cultivation. By the application of the proposed technique all the nutrients (K, P, N), which have been removed from the cultivation soil, could be recycled by using the produced biological fertilizer. By this way the sustainability of olive trees cultivation and of olive oil production renders feasible and the whole proposed technique proved to be effective and characterized as “clean” technology. This technology is already used by 4 olive mills in Greece with very promising results and can be a promising alternative either for all the olive mill owners or for olive trees and generally biological products cultivators.

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Guided discussion - Sustainable olive oil production: opportunities, obstacles and the path forward
S.E. Apitz, SEA Environmental Decisions Ltd

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Summary, conclusions and next steps
P. Masoni, ENEA / LCA and Ecodesign Laboratory
MO001
ORAC as a biomarker of antioxidant defense
S. Furuhagen, Stockholm University / Department of Applied Environmental Science ITM; B. Lieneworg, Stockholm University / Department of applied environmental science; M. Brotholz, Inst for tillamnd miljövetenskap / Department of Applied Environmental Science ITM; E. Gorokhova, Stockholm University / Department of Applied Environmental Science ITM
The antioxidative system is vital for organisms to maintain their oxidative homeostasis and protect biomolecules from oxidative damages. In ecotoxicological studies, alterations in the antioxidant defense is often assessed by assaying the activity of individual antioxidant enzymes separately. However, it is difficult to make any overall conclusions about effects on the antioxidant defense from these results as the enzymes may react differently from each other. The activity of a few selected enzymes may not provide a complete picture of the antioxidant defense as this is a complex system consisting of multiple enzymes and non-enzymatic compounds with antioxidative properties. However, there are several assays that measure the total antioxidative capacity in samples. In this study we look at one of these assay, Oxygen Radical Absorption Capacity (ORAC), as a biomarker of antioxidant defense in ecotoxicological studies. This assay has successfully been used to measure the levels of antioxidants not only in food and beverage, but also in biological samples, such as blood and plasma. There are also recent studies that have applied ORAC in ecotoxicological studies with invertebrates. In this study, we aim to establish a dose-response curve from ORAC as well as examine the time-dependent response of this biomarker. We also study how ORAC relates to the activities of constitutive antioxidant enzymes (e.g. CAT, SOD) and levels of lipid peroxidation. These relations are important to establish in strengthening ORAC as a suitable biomarker in ecotoxicological studies and to facilitate the interpretation of the ORAC response.

MO002
Gene expression analysis of antioxidant responses in Annelids, Brassicaceae and Fabaceae exposed to cadmium and/or lead contaminated soils.
F. BERNARD, LSVF, University of Lille / Laboratoire des Sciences Végétales et Fongiques Faculté de Pharmacie; F. Brulé, Unité Expertise in Toxicology Ecotoxicology of Substances DRC VIVA ETES; S. Dumez, University Lille2 / Laboratory of Plant and Fungal Sciences; S. Lemaire, Fonctionnement des Ecosystèmes Anthropisés; A. Platel, Institut Pasteur de Lille / Laboratoire de Toxicologie; F. Nessler, Institut Pasteur de Lille; D. Cuny, University of Lille 2; A. Daram, University of Lille 2 / Laboratoire des Sciences Végétales et Fongiques Faculté de Pharmacie; F. VANDENBULCKE, LGCgE, Université de Lille 1 / LGCgE EA Univ Lille Fonctionnement des Ecosystèmes Terrestres Anthropisés Cell and Quantitative Analysis of Eluvial Samples. Pollutants, such as Metal Trace Elements (MTEs) and organic compounds (polycyclic aromatic hydrocarbons, pesticides), can impact DNA structure of living organisms and thus generate DNA damage. Animal or vegetal cells use antioxidant defense systems to protect themselves against Reactive Oxygen Species (ROS) produced during oxidative stress. The mechanisms of production and management of these ROS were investigated in specific model ecosystems as unspecific markers. We performed a literature survey of the biochemical responses (enzymes activities) and expression variations of genes encoding proteins involved and/or related to antioxidant response in three species that are often used in ecotoxicology and that live in close contact with soil: the earthworm, Eisenia fetida, the white clover, Trifolium repens and the cabbage, Brassica oleracea. Literature survey and data search in molecular database were carried out on the basis of keywords in Scopus, in PubMed and in Genbank™ for each species (and in related species). In literature, some antioxidant enzyme activities have already been measured in those species but such analysis do not allow distinction of individual enzyme involvements in oxidative stress. Gene expression studies would allow this distinction at the transcriptional level. By exploiting the conservation observed between species and using molecular biology techniques, we partially cloned and measured the gene expression of many candidates involved and/or related to antioxidant responses in E. fetida, T. repens and B. oleracea. Therefore, analysis of the gene expression level of most effectors involved and/or related to antioxidant responses has been performed in the 3 species exposed in vivo (3, 10 and 56 days) in a well-characterised urban reference soil spiked with cadmium and/or lead at environmental concentrations. The use of gene expression analysis (in combination with metal accumulation analysis) has shown the interest of studying the effects of metal stress in several species. A major interest of this work lies in the measurement of most genes involved and/or related to antioxidant response at the same time and in several species. Indeed, as responses to oxidative stress may be different between species for the same stressor, one may keep in mind that model species should be used in a complementary manner in order to better understand the effects of xenobiotics on exposed organisms.
hazard identification and risk assessment. Recent evidence suggests that standard ecotoxicological assays may provide insufficient assessment of such microcontaminants and, hence, water quality. Previous remarks also suggest they should be better addressed by using sensitive biomarkers and adverse outcomes related to their mode of action (MOA). A good example, is that of selective serotonin reuptake inhibitors (SSRI), a group of widely consumed antidepressants, with which multiple different effects in aquatic species at low and high concentrations. For SSRI, disruption of fish behaviour, stress effects and metabolic pathways seem to be more sensitive endpoints than conventional testing based on standard species and growth and reproduction. Organisms of different backgrounds (e.g., genetic make-up, previous exposure history) may also exhibit differential sensitivity to contaminants, although this is seldom addressed. This study investigated acetylation and oxidation activities of SSSA from two estuaries with different contamination histories. A sub-chronic exposure to environmental and high sertraline levels was performed. MOA-related biomarkers were used to assess neurotransmission, energy metabolism, biotransformation and oxidative stress pathways. Besides non-monotonic responses, differing effects were elicited in test crabs, with differences in sensitivity of four orders of magnitude between the two estuaries. These included decreased acetylcholinesterase activity, indicative of ventilatory and locomotory dysfunction, inhibition of antioxidant enzymes and increased oxidative damage at ≥20.05 μg·L⁻¹. Crabs from a moderately polluted estuary also showed potentially life-threatening effects at concentrations about three orders of magnitude lower than those found for reproductive output in the most sensitive freshwater species tested up-to-date. Results point an influence of previous exposure history. This in exposure, in turn, in microbial volatiles used to assess toxicity of pharmaceuticals using Adverse Outcome Pathways and local potentially affected species. Funded by Project ECOEISK (NORTE-07-0124FEDER-000055) within the SR&TD Integrated Programme MARVALOR, through Programme ON 2 - O Novo Norte and the ERDF; and by the Portuguese Foundation for Science and Technology (PEst-C/ MAR/LA0015/2013).

**MO006**

**Effect of temperature and cypermethrin on the sentinel species Mytilus galloprovincialis measured through a suite of biomarkers in gills**

D. Ortiz, Universidade de Valencia / Dpt Biologia Funcional y Ambientologia Fisica; L. Yang, Spanish Research Council (CSIC) / Biology culture and pathology of marine species; M. Chiaramar, Universitat de Valencia; M. Sendra, Universitat de Valencia / Dpt Biologia Funcional y Ambientologia Fisica; A. Torreblanca, University of Valencia / Department of Animal Biology

Currently one of the biggest problems facing the salmon industry worldwide is high proliferation and spread of sea lice, copepod ectoparasites that reaches high proportion densities in salmon farms. The economic losses to the industry by reducing the health of the fish and indirectly by high treatment costs. In Chile the main insecticides used to lower the parasite loads through direct baths with skirts at sea are pyrethroids (cypermethrin and deltamethrin). In South Korea, organic farming materials are evaluated separately for plant growth and livestock control. This study was performed to evaluate the risks of the plant extracts used for pest control, by evaluating the toxicity to carp (Cyprinus Carpio) and muddy loach (Misgurnus anguillicaudatus) and calculating the estimated concentration in the exposed environment. The fish acute toxicity of plant extracts on carp and muddy loach were low in grade III/48h-LC50>2.0mg/L. The PEC (Predicted Environmental Concentration) using the plant extracts in paddy field of solphora extracts were 0.2–4.32 mg/L and neem extracts were 0.14–12 mg/L. The PEC of derivis extracts in river were 0.00036–0.0063 mg/L. The TER (Toxicity Exposure Ratio) using the toxicity values of muddy loach in paddy field of solphora extracts were 3.9–65.2 and neem extracts were 3.0–252.8. The TER using the toxicity values of carp in river of derivis extracts were 74 in 100% mortality. The estimated concentration of plant extracts were low for fish. In conclusion, when a company has to submit the organic farming material’s toxicity data for governmental registration and certification, among the plant extracts, solphora, neem and derivis extracts are desirable to skip the fish acute toxicity data.

**Behavior Revised: Examining Behavioral Effects of Contaminants and Other Stressors in Aquatic Animals (P)**

**MO007**

**Blood cholinesterase activity as biomarker for the diagnosis of organophosphate exposure in birds in chlorpyrifos treated pome fruit orchards in the UK**

M. Fouadouakis, Dow Agrosciences / RSRA ERS; S. Norman, RidgewayEco; F. Sotti, B. Giesing, Tier3 Solutions GmbH; N. Poliak, Dow Agrosciences / RSAACES; M. Smith, Huntingdon Life Sciences

The activity of cholinesterases, inclusive of both acetyl-cholinesterase and butyryl-cholinesterase (BuChE), has been commonly used as a biomarker for organophosphate (OP) exposure and risk in birds. Analysis of blood plasma cholinesterase (ChE) activity in field-caught birds allows OP exposure to be assessed non-destructively, allowing large numbers of individuals to be sampled, repeatedly, without impacting the local population. Pome fruit orchards in UK, treated twice each year with the OP insecticide chlorpyrifos (CHP), were selected in 2014 as sites to provide information on the status of bird communities, and to assess blood ChE activity in the same bird communities, providing a diagnostic evaluation of CHP exposure. Field searches showed the presence of different bird species, in all age stages, present before and after CHP application, with blue tit, great tit, blackcap, robin, chaffinch and blackbird. Blood samples were taken from approximately 300 individuals (adults and chicks) from 10 species during this study and plasma ChE activity of all species was determined using three ChE inhibitors (BW283C51, iso-OPMA and eserine). For all species studied, the main blood ChE form present was BuChE, and the range of BuChE activity that may be considered as basal levels for non-exposed individuals was determined. The results indicated that for all species BuChE activity was slightly decreased immediately after CHP application; the most prominent decrease was observed in blackbirds. For most cases, the BuChE activity completely recovered after a short period (approximately 10 days). In some species, e.g., blue and great tits, the plasma BuChE activity was higher in adults than in young birds. Moreover, detection of low level exposure, resulting from CHP application, by using plasma BuChE may be a better indicator of exposure than brain BuChE because the former is usually inhibited more rapidly and to a larger degree. The work undertaken in 2014 gave a good insight into potential exposure of birds utilizing the pome fruit orchards and showed a good correlation between the application of CHP and BuChE activity.
indicating recovery. These results showing increased mortality, locomotory impairment and inhibited feeding emphasize the strong toxicity of low, environmentally relevant concentrations of deltamethrin to non-target aquatic organisms and the synergistic effects of combination with imidacloprid on both lethal and non-lethal end-points.

MO010
Anti-anxiety drugs and fish behaviour: establishing the link between internal concentrations of oxazepam and behavioural effects

Benzodiazepines are pharmacologically designed to alter behaviour in humans by modulating the GABA<sub>A</sub> receptor and reducing the communication between neurons. This in turn leads to a calming effect in patients. Benzodiazepines have recently been detected in the aquatic environment and several studies have shown that the GABA<sub>A</sub> receptor is evolutionary conserved in aquatic organisms, including fish, suggesting that these compounds may elicit pharmacological activity in the environment. Among the different benzodiazepines, oxazepam has recently received considerable attention since some studies suggested that this compound may alter fish behaviour at environmentally relevant concentrations. Here we present the results of a study designed to establish the quantitative link between oxazepam and cyclooxygenase (COX) and eicosanoid (PGF<sub>2a</sub>) synthesis. This study was designed on the basis of the read-across hypothesis and was conducted by exposing fish to 1, 5, and 25 µg L<sup>-1</sup> of oxazepam for 28 days using a flow-through system. This range of oxazepam water concentrations was chosen to produce plasma concentrations below, equal and above the ones known to induce thrombo-inflammatory effects in humans. Oxazepam was quantified by LC-MS/MS in plasma, brain and liver of individual fish. Behavioural endpoints were quantified by performing two different tests - Novel Tank Diving Test and Shelter-Seeking test - after 14 and 28 days of exposure. Fish behaviour was analyzed blindly using automated video-tracking software. The full set of results will be presented and discussed together with the implications for the Environmental Risk Assessment of oxazepam.

MO011
Effects of cyclooxygenase inhibitor ibuprofen on prostaglandin synthesis and reproductive behavior in zebrafish
T. Firkus, D. Mandl, University of St. Thomas.

Prostaglandins are a class of hormones important for the regulation of reproductive behavior in zebrafish. Pharmaceuticals such as nonsteroidal anti-inflammatory pharmaceuticals (e.g., ibuprofen, IB) are designed to inhibit mammalian cyclooxygenase (COX) in different tissues (e.g., prosta-glandin) and their behavioural effects in the teleost family mormyrid (Pimelophales promelas). This study was designed on the basis of the read-across hypothesis and was conducted by exposing fish to 1, 5, and 25 µg L<sup>-1</sup> of oxazepam for 28 days using a flow-through system. This range of oxazepam water concentrations was chosen to produce plasma concentrations below, equal and above the ones known to induce thrombo-inflammatory effects in humans. Oxazepam was quantified by LC-MS/MS in plasma, brain and liver of individual fish. Behavioural endpoints were quantified by performing two different tests - Novel Tank Diving Test and Shelter-Seeking test - after 14 and 28 days of exposure. Fish behaviour was analyzed blindly using automated video-tracking software. The full set of results will be presented and discussed together with the implications for the Environmental Risk Assessment of oxazepam.

MO013
Visual and chemical cues - does EE2 exposure affect male mate choice of a freshwater fish?
M. Saaristo, R. Jacques-Hamilton, Monash University / School of Biological Sciences; M. Allinson, The University of Melbourne / School of Chemistry; M.G. Bertram, Monash University / Biological Sciences; C.P. Johnstone, B.B. Wong, Monash University / School of Biological Sciences

Aquatic habitats are increasingly being exposed to chemicals that, even at low levels, can disturb the endocrinology of organisms. One class of environmental contaminants posing a severe threat to freshwater ecosystems globally is that of endocrine disrupting chemicals (EDCs). Typically, research on EDCs has focused on physiological or morphological changes that can arise from exposure. Few studies have explored the effect of EDCs on behaviour. Among those that have, we know that behaviour can be affected at concentrations far lower than typically used to explore physiological or morphological changes. In this study, we examine the impact of EE2 exposure on larval perch (Perca fluviatilis) and damselfly (Calopteryx splendens) behaviour. EE2 exposure is commonly used in freshwater systems to test the endocrine disrupting potential of chemicals and to explore the potential of EDCs to disrupt perception processes. Exposure of perch and damselfly larvae following a short (7 days) exposure to an environmentally relevant concentration (5ng/L) of 17α-ethinyl estradiol (EE2) on male mate choice in the guppy. To do this we asked two questions: 1) Do males discriminate between exposed and control females when only presented with visual cues? 2) Do males discriminate between exposed and control females when presented only with chemical cues? To investigate the impact of EE2 on visual and chemical communication of guppies two behavioural experiments were employed: (1) a male was presented with a choice between the visual cues of an exposed and a control (i.e. non-exposed) female, and (2) a male was presented with a choice between the odour cues of an exposed and a control female. Both female and male behaviour were video-recorded. We found that EE2-exposed males spent significantly more time associating with exposed females when they could only use visual cues. On the contrary, when they were given only chemical cues to choose from, EE2-exposed males visited control female chemical cue more often than EE2-female cue.

MO014
Behavioral alterations following exposure to copper, phenanthrene, and copper-phenanthrene mixtures: linking behavior to acute toxic mechanisms in Daphnia azteca
P. T. Gauthier, Natural Resources Management; E.E. Prepas, Lakehead University / Department of Natural Resources Management; G.G. Pyle, University of Lethbridge / Biological Sciences

Phenanthrene (PHE) and copper are two contaminants commonly co-occurring in the environment. Among the different benzodiazepines, oxazepam has recently received considerable attention since some studies suggested that this compound may alter fish behaviour at environmentally relevant concentrations. Here we present the results of a study designed to establish the quantitative link between oxazepam and cyclooxygenase (COX) and eicosanoid (PGF<sub>2a</sub>) synthesis. This study was designed on the basis of the read-across hypothesis and was conducted by exposing fish to 1, 5, and 25 µg L<sup>-1</sup> of oxazepam for 28 days using a flow-through system. This range of oxazepam water concentrations was chosen to produce plasma concentrations below, equal and above the ones known to induce thrombo-inflammatory effects in humans. Oxazepam was quantified by LC-MS/MS in plasma, brain and liver of individual fish. Behavioural endpoints were quantified by performing two different tests - Novel Tank Diving Test and Shelter-Seeking test - after 14 and 28 days of exposure. Fish behaviour was analyzed blindly using automated video-tracking software. The full set of results will be presented and discussed together with the implications for the Environmental Risk Assessment of oxazepam.
marine and freshwater environments. Exposure to copper and PHE has been shown to alter the behaviour of fish and crustaceans. *Hyalella azteca* use pleopod appendages to ventilate the gills and promote respiration. Impaired ventilatory behaviour would reduce oxygen consumption, which would in turn effect a variety of additional behaviors (e.g., activity, swimming performance, and phototaxis).

Moreover, reduced oxygen consumption presents a potentially lethal physiological effect. Virtually nothing is understood regarding the effects of copper and PHE on the physiology of *H. azteca* and the mechanisms involved in oxygenation or respiration. Nonetheless, copper and PHEs are neurotoxic reactive produce oxygenated species (ROS). Accordingly, we explored the effects of copper, PHE, and copper-PHE mixtures on oxygen consumption, ROS production, acetylcholinesterase (ACHE) activity, and a suite of behavioral endpoints (i.e., activity, velocity, distance travelled, phototaxis, and pleopod beating). Our principle objective was to link behavioral alterations with oxygen consumption rates, ROS imbalance, and neurotoxicity in adult *H. azteca*. Adult amphipods (>6 weeks old) were used for all toxicity tests. Amphipods were exposed to 20 μg L⁻¹ copper, 200 μg L⁻¹ PHE, and a mixture of 20 μg L⁻¹ copper and 200 μg L⁻¹ PHE for 24 hrs. Immediately following the 24-hr exposure period, activity, distance travelled, velocity, phototaxis, and pleopod beats per minutes were measured. The same animals were then used to measure ROS levels using the dichlorofluorescin biomarker coupled with fluorescence microscopy. Oxygen consumption was measured via conventional closed respirometry, and ACHe activity was measured following Ellman’s method. Activity, distance travelled, velocity, phototaxis, and pleopod beating were all significantly and greatly reduced (e.g., 50% to 100% below control) following exposure to PHE. Whereas copper elicited a 40% increase above control, indicating that ROS imbalance is not responsible for behavioral alterations in *H. azteca* following acute exposures. ACHe activity assays are underway, and may provide a mechanistic explanation for behavioral impairment following exposure to PHE.

**MO015** Alcohol, coffee and cigarettes: evaluation of candidate positive control chemicals for behavioural assessment in zebrafish early life stages C. Di Paolo, RWTH Aachen University / Ecosystem Analysis ESA; J. Legradi, Institute for Environmental Studies; K. Graf, RWTH Aachen University; Department of Ecosystem Analysis ESA; R. Otermanns, RWTH University; L. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology; M. Fenske, Fraunhofer Gesellschaft IME / Ecotoxicology; J. Legler, VU University / Institute for Environmental Studies; H. Hollert, RWTH Aachen University / Institute for Environmental Research; T. Scoppettuoli, SCG/ RWTH Aachen University / Ecosystem Analysis ESA. The assessment of behavioural endpoints in zebrafish early life stages (ELS) can indicate organism-level responses to the most varied environmental contaminants. However, the procedures applied to evaluate behavioural changes are not clearly defined and vary a lot between laboratories and studies. This makes the interpretation and validation of experimental results very challenging. Behavioural assessment in zebrafish ELS would clearly benefit from the standardization of protocols. Therefore this study aimed to: investigate candidate positive control chemicals for behavioral testing with zebrafish ELS; and evaluate whether zebrafish ELS exposed to the same experimental procedures in two different laboratories would display comparable behavioural responses. The same set of experiments was performed at the Institute for Environmental Studies - VU University and at the Institute for Environmental Research - RWTH Aachen University. At VU, the automated video tracking system from ViewPoint was used; while at RWTH a custom-made system combined with the EthoVision software (Noldus) was applied. Zebrafish ELS were exposed to several concentrations of ethanol, caffeine and nicotine, and behaviour was studied at ≤ 5 days post fertilization (dpf). Long-term exposures between 0-5 dpf as well as short term exposures at 5 dpf (i.e, for 10 min, 40 min and 1.5 hr) were performed. Medium and solvent control conditions were included. Exposed zebrafish were submitted to light-dark short transition challenge, and video tracking of locomotion was performed for 24 cycles of transitions. Locomotion data was analysed for parameters as total distance moved, burst activity, and ratio dark:light activity. Statistical analysis concerning the concentrations of compounds, temporal patterns, and experimental setup. The intra and interlaboratory reproducibility between replicate experiments was evaluated. In our study, comparable behavioural patterns between laboratories were observed for the same compounds and exposure periods. However, some sensitivity differences were observed regarding compound concentrations. It is considered that positive controls might be useful to counteract these effects, by applying positive control conditions in adequate exposure scenarios. In general, our results clearly indicate the need and usefulness of interlaboratory comparisons, which can help to improve behavioural assessment in zebrafish ELS.

**MO016** Behavioural Effects of Coal Mine Wastewater in Australian Species C. Lantec, Central Queensland University / School of Medical and Applied Sciences; S.D. Melvin, Smart Water Research Centre / Centre for Environmental Management; L. Fabbro, Central Queensland University / School of Medical and Applied Sciences; F.D. Leusch, Griffith University / Smart Water Research Centre / School of Environment and Smart Water Research Centre; S. Wilson, CQ University / School of Medical / Applied Sciences

Coal mining represents a major economic activity, particularly so in Australia, but concerns about potential environmental effects necessitate effective monitoring strategies. Substantial volumes of mine wastewater may be discharged into the environment, through both planned and accidental releases. These discharges can be highly saline and may often contain high levels of dissolved solids, heavy metals, and hydrocarbons. Traditional toxicological testing has generally involved assessment of acute or chronic toxicity, and while such tests provide useful information, they are poorly suited to ongoing monitoring or rapid assessment following accidental discharge events. We therefore investigated behavioural responses of native Australian fish and cladocerans as a tool for evaluating potential sublethal effects of coal mine wastewater releases on freshwater ecosystems. Organisms were exposed to wastewater from two dams (0-100% CMW1 and CMW2) located at an open cut coal mine in Central Queensland, Australia. Behaviour was monitored using both EthoVision XT 9.0 video-tracking software (Noldus Information Technology, Netherlands) and the MultiSpecies Freshwater Biomonitor® (MBF, LimCo International GmbH). In experiments using the MBB, native fish (*Hyalella azteca*) exposed to coal mine wastewater showed a rapid reduction in time spent swimming and a slight increase in ventilation rates compared to controls. These effects were observed within 24 hours of exposure and persisted over the two-week exposure. Alternatively, in experiments using the EthoVision software, exposed *H. compressa* showed an increase in activity and spent more time near the arena border compared to controls after 48 hour exposure. Experiments suggest that the sensitivity of these tools for realistic environmental concentrations, and explore the practicality of these techniques for *in situ* monitoring.

**MO017** Can Psychiatric Pharmaceuticals Alter the Correlation Between Behaviours in Fish? A. Lagesson, Department of Ecology and Environmental Science; J. Klaiminder, Ecology and Environmental Science; J. Fick, Umeå University / Department of Chemistry; M. Jonsson, Department of Ecology and Environmental Science; T. Brodin, Umeå University / Department of Ecology and Environmental Science. Psychiatric drugs such as antidepressants and antipsychotics are a large group of pharmaceuticals that enter aquatic systems and remain biochemically active. A number of studies have demonstrated that psychiatric drugs can alter the behaviour of aquatic organisms, with possible consequences for aquatic ecosystems. However, there are many studies reporting that psychiatric drugs can alter correlations between behavioural traits, that are consistent within individuals over time and between contexts. Certain sets of behaviours show relationships (behavioural syndromes) that are consistent across ecological situations, suggesting limited behavioural plasticity. One way that psychiatric drugs might affect ecosystems is by modifying behaviour syndromes, with the potential to cause cascading effects on ecosystem functions. Knowledge regarding this is, however, currently insufficient. In this study, we hypothesised that psychiatric drugs can alter correlations between behavioural traits of wild fish. Our hypothesis was tested by synthesizing data from laboratory behaviour assays. The substantial data (n > 200), collected from wild caught individuals of European perch (*Perca fluviatilis*), revealed effects on two ecologically important behaviours: activity and sociability. We examined how these two traits were correlated before and after exposure to two different drugs: an antidepressant (Oxazepam) and an antipsychotic (Mirtazapin). We found that the correlation between the two studied behavioural traits was affected by Oxazepam and to a lesser extent by Mirtazapin. The findings imply that psychiatric drugs in surfaces waters have the capacity to not only modify single behavioural traits, but also the way in which different types of behaviours are related to each other.

**MO018** Antihistamines and aquatic insects: Bioconcentration and impacts on behaviour in damselfly larvae (*Zygoptera*) M. Jonsson, Department of Ecology and Environmental Science; J. Fick, Umeå University / Department of Chemistry; J. Klaminder, Ecology and Environmental Science; F. Finn, T. Brodin, Umeå University / Department of Ecology and Environmental Science

Aquatic insects use histamines as neurotransmitters and as wastewater are not cleansed from these chemicals it is reasonable to argue that they will be affected. In a study damselfly larvae was exposed to low concentrations of two commonly used antihistamines (*Hydroxyzine* and *Fexofenadine*, 360 ± 42 and 2200 ± 43 ng L⁻¹, respectively), and damselfly larvae behavior was recorded before and after exposure. After Hydroxyzine exposure, the damselfly larvae became less active, and they showed reduced fleeing response (i.e. increased boldness) after being exposed to Fexofenadine, the latter also being significantly different from the
non-exposed (control) individuals. We also found high levels of bioconcentration in the damselflies; Hydroxyzine showed an average bioconcentration factor (BCF) of 2000. Our results indicate that low concentrations of anthistaminines can have sub-lethal effects on aquatic insects manifested as behavioral changes, and that bioconcentration of these substances can be high. We, therefore, highlight the need to further investigate the impact these aquatic contaminants can have on ecologically relevant behaviors.

**MO019 Growth, Condition and Swimming Performance of juvenile Empire Gudgeons (Hypseleotris compressa) Following Short-term Exposure to Wastewater Treated in a Constructed Wetland**

S.D. Melvin, Smart Water Research Centre / Centre for Environmental Management; J.P. van der Merwe, Griffith University / Smart Water Research Centre; F.D. Leusch, Griffith University / Smart Water Research Centre / School of Environment and Smart Water Research Centre

There is growing interest into the use of Constructed Wetlands (CWs) for the treatment of domestic sewage, due to their relatively low operational costs and reported capacity to effectively remove nutrients, microbes and various emerging contaminants from wastewater. Throughout regional Australia, CWs are particularly important for wastewater treatment in rural and remote communities, where intensive treatment options are not practicable. Climatic conditions in Australia generally allow wetland operators to retain water during long dry periods, and to time discharges so that they coincide with peak rainfall events. As such, aquaculture and industry may adopt CWs as a part of their wastewater management strategy.

However, there is a need to continue to research the long-term ecological and socio-economic impacts of CWs. This project will focus on monitoring the growth and swimming performance of juvenile Empire Gudgeons (Hypseleotris compressa) exposed to wastewater from a CW. A range of different dilutions of CW wastewater will be used to determine the sub-lethal effects of wastewater on this species, which is native to the Shoalhaven River catchment. The potential for stable isotope techniques to be used as a biomonitoring tool will also be considered.

**MO020 Behavior, growth and biochemical responses of Gammarus pulex to prochloraz and fluoxetine exposure in a feeding experiment**

N. De Castro, Departament dEcologia. I. Muñoz, University of Barcelona / Department of Biology; A. Ferrer, University of Barcelona / Institute of Marine Sciences.

The development of toxicity tests based on single species responses has been widely proposed as a complementary tool to biological and chemical conventional surveys. Non-lethal endpoints are more sensitive than mortality or community structural changes and can be used as early warning indicators of toxic stress. The use of detritivores to detect specific effects of pollutants is currently becoming a well known method. Non-lethal endpoints are more sensitive than mortality or community structural changes and can be used as early warning indicators of toxic stress. The use of detritivores to detect specific effects of pollutants is currently becoming a well known method.

Aldous leaves were conditioned during 14d under three different treatments: 6 μg/L of prochloraz, equivalent to the LC50 values reported for algae; 100 ng/L of fluoxetine; and the mixture of both compounds. G. pulex adult individuals were fed during two weeks with the different treated leaf discs. In the feeding experiment the four conditions treated were: (control), 100 ng/L fluoxetine, 6 μg/L prochloraz and the mixture of both compounds were combined with two treatments: exposure to 100 ng/L of fluoxetine and control in order to study the direct effects of this pharmaceutical in the behaviour. Chlorophyll a, ergosterol and CNP levels of the leaves were analysed.

After the experiment, G. pulex behavioral responses to dark-light treatment were analysed with DanioVision, using Ethovision XT software. Preliminary results of G. pulex locomotive and feeding behavior after the experiment. The combination of these different endpoints might provide a useful tool for early detection of toxicity mixtures in freshwater ecosystems.
Locomotor velocity assay was used to monitor planarian mobility for a period of 3 min per organism. A new feeding inhibition test was developed where, chromidion larvae were added as food and feeding rate (n or larvae ingested) assessed for 24 h. Finally, planarian mobility was assessed using an automated video recording systems. This system allows an automated high throughput tracking of movements and covered distance of organisms. Our results indicate that Dugesia subterranea is a promising organism for bioassays, responding to insecticide stress after short-term exposure. Although D. subterranea attained higher tolerance to CAP compared to freshwater insects, behavior was significantly affected in exposed organisms. Planarians exposed to CAP showed a decreased locomotor velocity and feeding rate. This work intends to present different methods to assess behavioural effects of contaminants and other stressors on planarian behaviour. The aim of this work is to test the sensitivity of Dugesia subterranea for different experiments, under short-term and long-term exposure. The behaviour of these planarians will be discussed as well as the ecological relevance of behavioural endpoints in the context of trophic interactions, indirect effects of and ecological risk assessment of neurotoxic pesticides.

**MO024**

**Midge (Chironomus riparius)** and lamprey (Lampetra fluviatilis) larval behavior as indicator of sediment toxicity

J. Salmelin, University of Jyväskylä / The Department of Biological and Environmental Science; H. Hamalainen, University of Jyväskylä; A. Karjalainen, ECHA-European Chemicals Agency; M. T. Leppanen, Finnish Environment Institute / Laboratory Centre; H. Kivistö, National Institute for Health and Welfare / Department of Environmental Health; J.V. Kukkonen, University of Jyväskylä / Biological and Environmental Science; K. Vuori, Finnish Environment Institute / Syke Laboratories

Biological early warning systems, by measuring whether a stress response of an organism is within its normal range, alert rapidly when a toxicant is present in water or sediment. We evaluated if behavioural responses of chironomid and lamprey larvae could be used as indicators of toxicity of dioxins and mercury in sediments. We collected contaminated surface sediments from R. Kymijoki across a pollution gradient (six sites) with known history of industrial pollution, and from an external reference site (R. Uralpanjoki) not affected by industrial effluents. These field sediments were used in the laboratory exposures. First we analysed concentrations of polychlorinated dibenzodioxins, furans (PCDD/Fs), polychlorinated biphenyls (PCBs) and mercury (Hg) in the sediments. Also body burden of PCDD/Fs and PCBs were analysed from lamprey larvae collected from the study sites when present. We measured larval behavioral responses, locomotion and ventilation, via Multispecies Freshwater Biomonitor, which quantitatively records different behavioral patterns of animals by impedance conversion technique. Chironomid larval behavior was measured after a 10d exposure, and lamprey larvae at 0, 3, 7, 14 and 28d. Also mortality and chironomid growth were used as end points. Longitudinal contamination gradient in Kymijoki sediments ranged from the highest (8 420 ng g⁻¹ dw) PCDD/F concentrations in the upper section of the river to the lowest concentrations (370 ng g⁻¹ dw) near the river mouth. PCDD/Fs and Hg concentrations correlated strongly, the highest Hg concentration being in the upper river (1.2 µg g⁻¹ Edw). Body concentration of Hg increased with concentration of both PCDD/Fs and Hg over two orders of magnitude and of PCBs one order of magnitude higher in R. Kymijoki than in the reference river. Congener concentrations correlated strongly between the sediment and larval fat tissue of lampreys both for PCDD/Fs (Spearman ρ = 0.90, p < 0.001) and for PCBs (Spearman ρ = 0.86, p < 0.001). Chironomid growth was reduced in some of the contaminated sediments, but there were no differences in mortality amongst exposure groups, and these differences did not determine any consistent differences in behavioral responses of chironomids or lampreys between contaminated and reference sediments indicating that these two species might not be appropriate sentinel of sediment contamination by PCDD/Fs, PCBs and Hg.

**MO025**

**Impact of substrate colour on macrofouling**

M.A. Bighiu, Applied Environmental Science; A. Eriksson-Wiklund, Stockholm University / Applied Environmental Science; B. Eklund, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES

Biofouling of ships and leisure boats gives rise to many economic and environmental problems. Thus, there is a need for predictive measures, most commonly in the form of antifouling paints. Nevertheless, there are several well-known physical factors that influence the biofouling process, such as temperature, water currents, substrate colour and texture, etc. The aim of our study was to investigate the role of substrate colour on the settlement of different macrofouling species. While most other studies used painted panels for this kind of exposure, i.e. black, white, blue and red (N=35 for each colour) as well as transparent panels (N=22) that served as ‘substrate controls’. The panels were attached on PVC bars and immersed in water, in vertical position, at a depth of 1.5 m for 21 days, after which the macrofouling species were identified and the dry biomass was determined. The results show that different species have different preferences for substrate colours. Thus, barnacles (Balanus sp.) settled predominantly on darker colours while mussels (Cerastoderma sp.) were most abundant on blue and transparent panels. These results show that the colour of the substrate is an important factor in the biofouling process and it should be taken into consideration in antifouling paint formulations. Therefore, choosing lighter colours for coating boat hulls can reduce macrofouling to a certain extent.

**MO026**

**Effects of silver ions and nanoparticles on zebrafish (Danio rerio) larvae behaviour**

G. Asmonaitė, K. Bresolin de Souza, University of Gothenburg / Department of Biological and Environmental Sciences; L. Forlin, University of Gothenburg; J. Sturve, Goethe University / Zoophysiology Dept

The assessment of fish behaviour is gaining more recognition in ecotoxicological research due to its high sensitivity compared to conventional LC₅₀/screenings and its capacity to reveal the effects of the whole suite of environmental stressors. Therefore, the aim of this study is to investigate the effect of silver ion exposure on the locomotor activity of zebrafish larvae. The behavioural endpoints have a great potential to capture complex biochemical and physiological consequences exerted by toxicant exposure on the organism level and can be used as a screening tool to assess the health status of exposed animals. As it has been previously reported, silver can potentially affect the neurodevelopment and cause behavioral retardations in early life stages of zebrafish (ZF). In this study, we exposed ZF embryos to a wide range silver (Ag⁺ and AgNPs) concentrations and evaluated developmental toxicity according to standardized FET (Fish Embryo Toxicity) test criteria. The locomotion activity of ZF larvae was quantified at 24 hpf via View Point® Video tracking system using a customized behavioural assay. A set of repeated episodes of light/dark stimulation was used to artificially stress and evoke visual motor responses, which were used as endpoints for sublethal toxicity. From this study, we were able to draw a conclusion that OSPW significantly inhibits the feeding behaviour, suppresses the growth and reduces the reproductive output of D. magna. These results are consistent with the hypothesis that OSPW may affect ecological function through bottom-up trophic cascades.
Environmental Chemistry
As the anti-cancer drugs allow people to live longer with higher standards of living, the consumption of such compounds will increase and consequently their presence in the drinking and wastewater is also expected to rise. Imatinib (IM) is a cytostatic compound that was developed during the 1990s and has since then been used in large scale in the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors. This study investigated the photodegradation of IM under UV and simulated sunlight irradiation as well as its biodegradability and toxicity before and after the treatments. An UV polychromatic lamp and a Xe lamp (simulating the sunlight spectrum) were used to perform the photodegradation experiments in a photoreactor containing 600 mL of a solution of IM at an initial concentration of 20 mg/L and prepared with ultrapure water. Irradiation was done for 120 min. After the treatments, the samples were subjected to biodegradation and ecotoxicity assays. The biodegradation experiments followed the guidelines of the OECD Closed Bottle Test (CBT), whereas the ecotoxicity assessment was carried out in accordance to a new automated method with the bioluminescent bacterium Vibrio fischeri which allows for the determination of the acute, chronic and growth toxicities (Menz et al., 2013). According to the biodegradation experiments IM has been classified as a non-biodegradable compound (< 8 %). The results showed that the parent compound IM did not underwent photolysis with the Xe lamp and was only a partially eliminated when using the UV lamp (48%). Furthermore, no significant mineralization (< 3%) was observed after the experiments performed with both lamps. Not surprisingly no differences were observed regarding the biodegradation and toxicity of the samples after being submitted to the irradiation with the Xe lamp, since no primary microbial activity was carried out under a treatment time of 250 min. The partially elimination of the parent compound when using the UV lamp did not improve the biodegradability or reduce the toxicity of IM. In conclusion, our study showed that other treatment options should be tested in order to eliminate IM and in this way improve its biodegradability and reduce its toxicity.

MO029
Using a freshwater clam valve rhythm dynamics-based novel valvometry technique to detect waterborne compounds
K. Kuemmerle, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry; E. Baginska, Leuphana Universität Lüneburg; L. Machado, Institute of Sustainable and Environmental Chemistry
L. Jou, National Ilan University; B. Chen, MingDao University; W. Chen, National Taiwan University / Dept Bioenviron Sys Eng; C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering
The purpose of this study was to use an improved valvometry technique as a detection system to effectively measure waterborne copper (Cu) based on valve activity dynamics in freshwater clam Corbicula fluminea. We adopted the magnitudes of shell gape of 20% and 50% as the determining thresholds of valve closing and siphon extension statuses, respectively, to digitalize valve movements in bivalves for quantifying the characterization of valve behavioral rhythmic responses. The improved valvometry technique allows tested freshwater bivalves with a free-range spontaneous situation to avoid stresses from experimental artefacts, increasing their measurable sensitivity and reducing probability of abnormal closings. With reliable statistical modeling approaches, we linked proposed valve daily rhythmic models and the toxidynamics-based Hill model to predict valve dynamic responses under different Cu exposures with a circadian valve rhythmic endpoint. Behavioral toxicity assays revealed Cu detection thresholds of 5.6 (95% CI: 2.1–9.3) mg l\(^{-1}\) and 19.5 (95% CI: 14.6–24.3) mg l\(^{-1}\) for response times of 300 and 30 min, respectively. Our findings reveal that when exposure Cu concentrations exceed 50 mg l\(^{-1}\), the differential degree of valve rhythmic response has a significant sensing ability within 30 min. The observation results demonstrate that our modeling approaches have robust prediction effects on describing valve rhythmic response variations. Our results implicate an early warning dynamometric biomonitoring system that provides a potential tool in situ detection of waterborne Cu concentrations by circadian valve activities in C. fluminea, making it suitable for distinguishing the safe water resources in the site-specific areas.

MO030
Mechanistic considerations of ozone-amine reactions
A. Tekle-Roettering, Westfälische Hochschule
Agnes Tekle-Röterting, Winfried Schmidt, Torsten C. Schmidt
Micropollutants in drinking water and wastewater treatment, micropollutant abatement by ozonation is gaining importance. There is, however, now also growing interest in the ozonation by means of Ozonation. In the present study the mechanism of ozone with anilines, nitrogen-containing aromatic heterocycles and nitrogen-containing aliphatic heterocycles as well-water-soluble amines are compared. Ozone reacts strongly electrophilic and highly selective and one of the ozone-reactive site is the lone electron pair at nitrogen, which has to be accessible. Protonation reduces the otherwise high ozone reactivity and so reactivity depends on pH. A second potential site of ozone attack is the electron-rich aromatic ring (von Sonntag and von Gunten, 2012). In literature, several primary ozone reactions with miscellaneous compounds have been discussed (von Sonntag and von Gunten, 2012), so that miscellaneous conceivable primary processes (in reactions 1-5 a few examples are presented) can be suggested for the ozone-amine- (aqueous or aromatic ring plus additional H-abstraction) on the nitrogen (reaction 3) in the case of anilines and N-containing aliphatic heterocycles or at the aromatic ring in the case of anilines and N-containing aromatic heterocycles. The resulting highly reactive intermediates, like ammien radicals, nitroxy radicals, hydrotrioxide radicals, hydrogen peroxy radicals, amino radicals cations or ozoneide radicals will undergo different reactions and the products that are formed in the ozone-amine reactions may indicate the initial mechanism. To what extent the miscellaneous reactions proceed, is reflected in ozone rate constants, ozone consumption and product yields and in addition was assessed by quantum-chemical calculations.

MO031
Assessment of the biodegradability and ecotoxicity of the anti-cancer drug Imatinib mesylate after UV and simulated sunlight irradiation treatments
C.A. Lutterbeck, Institute of Sustainable and Environmental Chemistry Faculty of Sustainability, E. Baginska, Leuphana Universität Luneburg; L. Machado, Institute of Sustainable and Environmental Chemistry; K. Kuemmerle, Leuphana Universität Lüneburg / Institute of Sustainable and Environmental Chemistry
As the anti-cancer drugs allow people to live longer with higher standards of living, the consumption of such compounds will increase and consequently their presence in the drinking and wastewater is also expected to rise. Imatinib (IM) is a cytostatic compound that was developed during the 1990s and has since then been used in large scale in the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors. This study investigated the photodegradation of IM under UV and simulated sunlight irradiation as well as its biodegradability and toxicity before and after the treatments. An UV polychromatic lamp and a Xe lamp (simulating the sunlight spectrum) were used to perform the photodegradation experiments in a photoreactor containing 600 mL of a solution of IM at an initial concentration of 20 mg/L and prepared with ultrapure water. Irradiation was done for 120 min. After the treatments, the samples were subjected to biodegradation and ecotoxicity assays. The biodegradation experiments followed the guidelines of the OECD Closed Bottle Test (CBT), whereas the ecotoxicity assessment was carried out in accordance to a new automated method with the bioluminescent bacterium Vibrio fischeri which allows for the determination of the acute, chronic and growth toxicities (Menz et al., 2013). According to the biodegradation experiments IM has been classified as a non-biodegradable compound (< 8 %). The results showed that the parent compound IM did not underwent photolysis with the Xe lamp and was only a partially eliminated when using the UV lamp (48%). Furthermore, no significant mineralization (< 3%) was observed after the experiments performed with both lamps. Not surprisingly no differences were observed regarding the biodegradation and toxicity of the samples after being submitted to the irradiation with the Xe lamp, since no primary microbial activity was carried out under a treatment time of 250 min. The partially elimination of the parent compound when using the UV lamp did not improve the biodegradability or reduce the toxicity of IM. In conclusion, our study showed that other treatment options should be tested in order to eliminate IM and in this way improve its biodegradability and reduce its toxicity.

MO032
Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities
A. Barra Caraciolo, National Research Council / Water Research Institute; E. Topp, Agriculture and Agri-Food Canada (AAFC); P. Gremini, National Research Council of Italy (CNR) / Water Research Institute
Introduction: Large amounts of pharmaceuticals are discharged into the environment per year and would undergo chemical and/or microbial transformations. However, the presence of these pharmaceuticals and their transformation products for risk assessment...
College

The effects of antidepressants of wildlife are currently raising some concern due to an increased number of publications indicating biological effects at environmentally relevant concentrations (<100ng/L). These results have been met with some skepticism due to the higher concentrations required to detect effects in some species and the perceived slowness to therapeutic effects recorded in humans and other vertebrates. Since their mode of action is to modulate the neurotransmitters serotonin, dopamine, and norepinephrine, aquatic invertebrates that possess transporters and receptors sensitive to activation by these pharmaceuticals are potentially affected by them. We highlight studies on the effects of antidepressants, on particularly crustacean and molluscan groups showing they are susceptible to a wide variety of neuroendocrine disruption at environmentally relevant concentrations (μg/L). Some effects observed in these species can be observed within hours of exposure. For example, exposure to amphipod crustaceans to several selective serotonin reuptake inhibitors (SSRIs) can invoke changes in swimming behaviour within hours. In molluscs, exposure to SSRIs can induce spawning in male and female mussels and foot detachment in snails within several hours of exposure. In the light of new studies indicating effects on the human brain with just dose of SSRIs using MRI scans, we discuss possible reasons for the discrepancy in former results in relation to the “read-across” hypothesis, variation in biomarkers used, phylogenetic distance, and the affinity to different targets and differential sensitivity to receptors.

MO307

Pharmaceuticals ecotoxicity: data curation and QSAR modeling

A. Sangiog, DiSTa; S. Cassani, P. Gramatica, University of Insubria / DiSTa

In the last decades pharmaceuticals have become a class of emerging potential pollutants since they are increasingly present in waste and surface water, air and soil. Due to their ubiquitous present in the environment, the European regulatory agency on pharmaceuticals, EMEA (European Medicines Evaluation Agency), published, in 2006, a guideline for the environmental risk assessment of human pharmaceuticals that shall accompany the application for marketing authorisation. Like every risk assessment procedure, this guideline requires, for each chemical, a large amount of data as logKow, ecotoxicity, environmental fate, consumption and so on. Unfortunately ecotoxicological data are available only for a little percent of the pharmaceuticals and literature databases looking for consistent ecotoxicological effects based on quantitative structure–activity relationships (QSARs) are valuable tools to maximize the information contained in existing experimental data and to predict missing information. However the quality and predictivity of a model depends on the quality of the data input according to the general rule of computer science GIGO (Garbage in, Garbage out). We checked big online databases like ECOTOX or OECDSARs and literature databases looking for consistent ecotoxicological effects based on quantitative structure–activity relationships (QSARs) are valuable tools to maximize the information contained in existing experimental data and to predict missing information. However the quality and predictivity of a model depends on the quality of the data input according to the general rule of computer science GIGO (Garbage in, Garbage out). We checked big online databases like ECOTOX or OECDSARs and literature databases looking for consistent ecotoxicological effects.
other contributing factors to toxicity than Dow, particularly for green algae and blue-green algae. Adequate data to calculate both acute and chronic PNEC values were available for 57 of the APIs. For most compounds, PNECs calculated from acute data were lower than PNECs calculated from chronic data. Five of the 57 PNECs (9%) calculated from acute data were below the EU action limit of 0.01 ppb and all were classified as anti-infective affecting algal species. Six of the 89 (6.7%) acute PNECs calculated from chronic data were below the EU action limit and for all of these APIs fish was the most sensitive species. Analysis of the data suggests that the use of acute data to determine chronic PNECs are acceptable if chronic data are not available, unless specific mode of action concerns suggest otherwise (e.g. the API has known or suspected endocrine active properties).

iPIE: Intelligence-Led Assessment of Pharmaceuticals in the Environment

A. Boxall, University of York / Environment Department; R. Laenger, Bayer Pharma AG / Dept Experimental Toxicology; A. Coors, ECT Oekotoxikologie GmbH; J.R. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; F. Sanz, IMIM

A variety of human pharmaceuticals have been detected in the natural environment across the world. Although reported concentrations are generally low, many pharmaceuticals have been detected in a variety of hydrological, climatic, and land-use settings and some can persist in the environment for a significant time period. As these are biologically active compounds that are designed to interact with specific pathways and processes in target humans and animals, concerns have been raised about the potential effects of pharmaceuticals in the environment - and over the past 15 years, a substantial amount of work has been done to determine the occurrence, fate, effects, and resulting risks of pharmaceuticals in the environment. Regulations have also been developed regarding the assessment of environmental risks of new pharmaceuticals within the market approval procedures. Currently, environmental assessments of pharmaceuticals are typically performed during the early development phases and allow for an application for market approval. The identification of potential environmental concerns at such a late stage can merely describe the potential for environmental impact but cannot influence any early stage development or identification of specific targeted assessment strategies. Furthermore, concerns have been raised over whether the standard testing methods for examining chronic effects on organisms will identify ecologically important effects of specifically acting pharmaceuticals. There is a need to address the legacy of existing APIs - more than 3,000 pharmaceuticals are currently in use today on the environmental risks is only available for a small proportion of these. iPIE is a 9.8 M Euro EC project, involving leading researchers from academia and the pharmaceutical industry, that aims to develop a framework (using information from toxicological studies, pharmacological mode of action and predictive models) for identifying the potential risks of human pharmaceuticals to the natural environment (considering both aquatic and terrestrial systems, including top predators). In the future this framework should be used to better target environmental testing requirements at an early stage in drug development programmes and for prioritising legacy pharmaceuticals for experimental testing and monitoring.

Post-Approval Environmental Stewardship of Tamoxifen

K. Hutchinson; R. Murray-Smith, AstraZeneca; J.R. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment

Tamoxifen is an estrogenic drug that is widely used to treat breast cancer. Tamoxifen is an established product that pre-dates current EU regulatory requirements for environmental risk assessment (ERA). It is highly metabolized in the liver, and both tamoxifen and its metabolites are excreted and released into the aquatic environment. Through our post-launch Ecopharmacovigilance initiative we have collated data points and information from AstraZeneca studies and published literature on the effects of tamoxifen on a range of aquatic species, together with information on the predicted fate and exposure of tamoxifen and measured concentrations in various environmental media. This poster will present a comprehensive summary and analysis of all the published data available for tamoxifen and an assessment of the environmental risks.

Monograph System for environmental data of active pharmaceutical substances: necessity, challenges and perspectives

J. Rönnefahrt, Federal Environment Agency / Section IV Environmental Risk Assessment of Pharmaceuticals

Even more than in the past, implementing the environmental risk assessment (ERA) into the European pharmaceutical legislation there is still a lack of data on fate and effects of active pharmaceutical substances. The reason for that is quite simple: A review program of ‘legacy’ products, which were approved before the ERA requirement was set, was never envisaged. In the pre-market phase an environmental risk assessment is required for all new applications. This leads to duplication of data and of assessments. Due to the fact that results of fate and effect studies may vary, this may also lead to contradictory assessments and even to different risk mitigation measures for medicinal products intended for the same indication. This is not acceptable, neither from a scientific nor from an economic point of view. Furthermore, the described problems with the functioning of the current legislation could largely be solved with the establishment of a monograph system on active pharmaceutical substances. The aim of a monograph system is to collate existing fate and effects data on active pharmaceutical substances, to generate missing data, to evaluate studies and to agree on the endpoints to be used for ERA of medicinal products. Once established all authorizations of medicinal products would use the agreed endpoints from the monograph of the respective active substance in order to perform the environmental risk assessment of the product. A monograph on fate and effects data would have a dual role representing the efficiency of the authorization procedure of medicinal products. Overall, only a pre-market monograph system in conjunction with an effective monitoring within the post-market surveillance will be able to ensure the environmental safety of veterinary medicinal products in use. Environmental data collated in monographs should primarily be adapted to the specific target receptor of a compound and be based on specific targeted assessment strategies. This is essential to ensure up-to-date risk assessments of veterinary medicinal products. Therefore monographs on active pharmaceutical substances will be the key measure to ensure the environmental safety of veterinary medicinal products in use. Challenges for the establishment of a monograph system are first of all clear rules on set up and management of consortia, rules on data protection, cost sharing etc. Examples of successful collaborations already exist in other legislative areas like REACH and biocides.

Environmental risk assessment of pharmaceuticals and endocrine disruptors in surface waters of the State of São Paulo (Brazil)

C. Lopes, University of São Paulo / Experimental data. Environmental risk for pharmaceuticals was detected in two studies for algae and crustaceans. HQ values ranged from 0.002 to 585. Risk for EDCs was presented in only 2 studies, but showing high possible estrogenic activity. The papers used in this work, reported high concentrations of pharmaceuticals and EDCs in freshwater ecosystems, maybe due to the lack of advanced sewage treatment and uncontrolled spillage from veterinary medicines. The studies present data for a number of pharmaceuticals and EDCs detected in Brazilian freshwaters. Further in situ studies should be developed to confirm deleterious effects on biota.

Pharmaceuticals in the environment: global occurrence, effects, and potential cooperation on action


The poster presents results of an extensive literature survey on the global occurrence of pharmaceuticals in the environment. Data on measured environmental occurrences from more than 1,000 international publications have been transferred to a database, with more than 120,000 entries. According to the database, pharmaceuticals have been transferred to a database, with more than 120,000 entries. According to the database, pharmaceuticals have been detected in the environment worldwide in all five UN regions. Most published data are for the compartments surface water and sewage effluent; less information is available for groundwater, manure, soil, and other environmental matrices. More than 600 active pharmaceutical substances (or their metabolites and transformation products) have been detected in the environment. Most findings have been published for industrialized countries. Monitoring programmes are increasingly being conducted in developing and emerging countries. These have revealed the global scale of the occurrence of pharmaceuticals in the environment. For example, diclofenac, a non-steroidal inflammatory drug, has been detected in the aquatic environment in 50 countries worldwide. A number of globally marketed pharmaceuticals have been
found in both developing and industrialized countries. The available ecotoxicological information indicates that certain pharmaceuticals pose a risk to the environment at measured concentrations. Options for cooperative action to address the risk of are also presented. The aim of the research presented was to support the discussion of the proposed emerging policy issue pharmaceuticals in the environment under the Strategic Approach to International Chemicals Management (SAICM), which is a global initiative of United Nation Environment Programme (UNEP).

MO044
Prioritisation of pharmaceuticals and metals - literature vs laboratory
K. L. Donnachie, E. Gozzard, Centre for Ecology and Hydrology; J. Ojogboro, Brunel University; M. D. Scriminow, Brunel University / Institute for the Environment; A. C. Johnson, CEH Wallingford / Wallingford; J. P. Sumpter, Brunel University / Institute for the Environment

Thousands of pharmaceuticals are entering our aquatic environment every day but which ones pose the greatest risk? We do not have the time or resources to assess the environmental risk from every pharmaceutical to the level of detail we would like. This forces us to consider different prioritisation approaches. This study has the long term aim of prioritising all chemical groups on the basis of risk through analysis of the literature. Twelve key pharmaceuticals were identified by selecting those most frequently cited by the scientific community in literature risk rankings using such factors as annual consumption, persistence, potency and predicted fish plasma concentrations. The twelve, which emerged most frequently, were Ibuprofen, Duloxetine, Paracetamol, Carbamazepine, Naproxen, EthinyLstradiol (EE2), Propanolol, Atenolol, Sulfamethoxazole, Metoprolol, Ofloxacin and Fluoxetine. These twelve pharmaceuticals were ranked by risk through comparing all literature ecotoxicity effect values with those either reported or predicted in UK water courses. As part of the greater study, this same approach was also used for the metals although in this case a representative sub-set of the ecotoxicity literature was used. The ranking compares the median ecotoxicity concentration with the median river water concentration. Overall, the metals emerged as a greater risk to aquatic wildlife than the pharmaceuticals. The pharmaceutical identified as the greatest risk using this approach was EE2. As a reality check on this literature based risk-ranking analysis, the toxicity of selected metals and pharmaceuticals were compared in our laboratory using Pseudokirchneriella subcapitata and Daphnia magna. The ranking using this laboratory methodology largely matched the ranking using the literature analysis. In theory, the ranking of these selected chemicals on the basis of toxicity to an alga and daphnid should match that from the literature survey. Here we will report how the two compared.

MO045
Risk based prioritization of human pharmaceuticals in Korean water environment
K. Ji, Yongin University; E. Han, Seoul National University; J. Park, SOON CHUN HYANG UNIVERSITY / College of Natural Sciences; J. Ryu, National Institute of Environmental Research / Risk Assessment Division; K. Choi, Seoul National University / School of Public Health

Pharmaceuticals have been used for protection of human and animal health, however their residues in the aquatic environment are potential threat to ecosystem health. In order to identify high priority pharmaceutical residues in terms of ecological risks, several prioritization schemes have been proposed to date. However, most of previous approaches are focused on the level of environmental occurrences or the amount of use. We proposed a prioritization strategy that considered both the levels of environmental occurrence and the potential hazards of the human pharmaceuticals in Korea. A total of 695 human active pharmaceutical ingredients (APIs) were first screened by logKow, annual production volume, and predicted environmental concentrations (PECs). Annual production volume of the target APIs were derived from drug production statistics of Korea as of 2011. PECs were derived in Han River of Korea by use of pharmaceutical assessment and transport evaluation (PASTE™) program. In addition to the number of pharmaceuticals screened for high ambient occurrence levels, major antineoplastic drugs and hormones were included in the initial list because of their high potential of toxicity. Then, the final lists of priority pharmaceuticals were derived based on the hazard quotient of each APIs. Probucol (logKow>3.5), tamoxifen (an antineoplastic drug), testosterone (hormone) and fluoxetin (synthetic hormones) were selected as final lists. Because of their low or often seemingly negligible levels of occurrence in ambient water, these pharmaceuticals have neither been adequately monitored nor assessed for risks. Further efforts are warranted to understand their risk potentials in the aquatic environment of Korea.

MO046
How to prioritise veterinary pharmaceutical substances for a monograph system?
J. Ebert, Umweltbundesamt / Pharmaceuticals Department; A. Hein, Federal Environment Agency (UBA) / Section IV Pharmaceuticals

The poster presents a new and manageable prioritisation concept for veterinary pharmaceutical ingredients (VPIs) intended for inclusion in a currently discussed monograph system as a starting point for further discussions. There are 400-600 active veterinary substances on the European market. Only a limited amount of these are environmentally relevant and must be considered in the intended monograph system for VPIs. Therefore, it is necessary to prioritise. Our prioritisation concept aims to identify the substances that have the highest potential environmental risk and/or the highest potential presence in the environment. We propose a simple, three-step method, which is based on the EMA/VICH Guideline for the environmental risk assessment of veterinary pharmaceuticals considering substance specific information on consumption, exposure, fate and effects available to a German regulatory authority. The proposed method classifies the substances into 4 priority categories, which reflect the potential environmental relevance and therefore the priority for inclusion in the monograph system. This work was to determine few and relatively simple criteria for a rough classification of active ingredients for which monographs should be compiled first. Prioritisation, exemplarily done on the basis of data available for Germany has shown that most of the active substances are expected to be in class II (high priority) and class III (manageable priority). The majority turn out to be the environmentally most relevant substances. The suggested method is considered to be transferable to other EU countries because all base data necessary for prioritisation should be available to other national regulatory authorities as well.

MO047
Prioritization of veterinary antibiotics for higher-tier environmental fate and effect analysis using a simple screening approach
J. Menz, J. Müller, M. Munck, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry; S. Gottschalk, Leuphana University; K. Kuenemmer, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry

Vertenoevermicrbiotic drugs (VAs) are widely recognized as important environmental contaminants and are frequently detected in environmental samples throughout the recent years. The environmental occurrence, fate and effects of VAs are strongly dependent on varying local factors, which is why region-specific priorities for environmental risk assessment (ERA) should be established. Moreover, substance prioritization in ERA of VAs is usually based on predicted or measured environmental concentrations, whereas the potential hazards of VAs are often not considered. In this context, a more integrated screening procedure was developed and exemplarily applied in a case study of northwestern Germany. The first level (A) included a comprehensive usage pattern-based exposure screening (UPEAS) to identify environmentally relevant substance candidates on the basis of VA consumption data. The identified candidates were subjected to a biodegradability screening (level B) using a modified closed bottle test (CBT), which allows identifying false negative results due to antimicrobial effects of the test substance with a considerably improved reliability compared to the standard CBT (OECD 301D). Finally, the toxicity towards soil bacteria (level C) of non-readily biodegradable VAs was evaluated in a modified contact assay for the long-term exposure. After screening level A, 7 VAs (Aminocillin, Chlorotetracycline, Colistin, Enrofloxacin, Sulfadiazine, Tetracycline and Trimethoprim) were identified as high-priority substances. These candidates were further classified as not readily biodegradable or highly toxic to heterotrophic microorganisms in screening level B and hence might persist in the environment in an active form. Therefore, the toxicity towards the soil bacteria species Bacillus subtilis and Bacillus amyloliquefaciens of selected substances was assessed in screening level C, which revealed strong variations between the analyzed compounds in terms of antimicrobial activity, species selectivity and bioaccessibility in presence of bulk soil. With this simple screening approach we were able to prioritize VAs under consideration of the regional environmental relevance, biodegradability and intrinsic antimicrobial activity. Therefore we envisage that the selected VAs could be a target for future research. In future studies, spatial distribution should be considered as well.

MO048
Ecotoxic and genotoxic effects of last-resort and broad-spectrum antibiotics - fate and behavior of potential future micropollutants in aquatic systems
J. Brunings, W. Dott, RWTH Aachen University / Institute of Hygiene and Environmental Medicine; H. Hollert, RWTH Aachen University / Institute for Environmental Research; J. Blessing, Institute of Environmental Engineering, RWTH Aachen / Institute of Hygiene and Environmental Medicine

Pharmaceuticals are yet to be evaluated for their aquatic ecotoxicity and bioaccessibility in presence of bulk soil. With this simple screening approach we were able to prioritize VAs under consideration of the regional environmental relevance, biodegradability and intrinsic antimicrobial activity. Therefore we envisage that the selected VAs could be a target for future research. In future studies, spatial distribution should be considered as well.
substances in aqueous systems was determined with the combined and optimized test system ‘O₂/CO₂-Headspace Test’ with GC-TCD following standard procedures (ISO) with modifications for measurement of the ultimate aerobic mineralization of test substances to carbon dioxide in water. The last-resort antibiotic Tigecycline was evaluated as ‘good (ready) biodegradable’, since degradation was exceeded 60% within 28 days. In contrast Linezolid was non-biodegradable. There were no ecotoxic effects for the broad-spectrum antibiotic Doripenem. The antibiotic Tigecycline showed the highest ecotoxicity in the algae test (EC₅₀ 3.4 mg/L) with maximum test concentration of 200 mg/L. For the daphnia immobilization assay Linezolid showed highest toxicity (EC₅₀ 160.4 mg/L). Contrary to ecotoxicity there was no genotoxicity for Linezolid. Genotoxicological effects were detected for Doripenem and Tigecycline with and without metabolic activation ranging from 1 to 100 mg/L.

MO049
In vitro metabolism and metabolite identification of select antibiotics, propranolol and diclofenac by rainbow trout (Oncorhynchus mykiss) liver S9 fractions
M. Dedajda; K.A. Connors, US Environmental Protection Agency / ORDNCT; A.J. Ramirez, Baylor University / Baylor Science Facility; C.K. Chambliss, Baylor University; P. Fitzsimmons, U.S. EPA; J.W. Nichols, U.S. Environmental Protection Agency; Q.B. Cass, Universidade Federal de São Carlos / Quimica; B.W. Brooks, Baylor University / Dept of Environmental Science

MO050
Effect of erythromycin exposure in Oncorhynchus mykiss gills and liver: detoxification mechanisms, oxidative defence system and peroxidative damage.
S. Rodrigues, Oporto University / Biology; S. Antunes, University of Oporto / Biology; C. Moreira, CIMAR / University of Porto; A.T. Correia, CIMAR and Faculdade de Ciências da Saúde da Universidade Fernando Pessoa; B. Nunes, CESAM / University of Aveiro

The increasing presence of pharmaceuticals in the aquatic environment, is cause of concern due to the occurrence of deleterious effects, including oxidative stress, in non-target species. Erythromycin (ERY) is a macrolide antibiotic, which is widely used as an antibiotic agent. ERY is widely used as human therapeutic agent to treat infections, and also in aquatic organ, usage, ERY was already detected in surface waters, in levels between several ng/L to µg/L. Recent studies have indicated unintended biological activity of ERY on non-target organisms, at different levels of biological organization. The present study aimed to assess the toxic effects of ERY in Oncorhynchus mykiss exposed during 28 days, under realistic levels of exposure (0.05 – 0.80 µg/L). In order to evaluate the ERY induced rainbow trout (Oncorhynchus mykiss) with biochemical markers, such as catalase (CAT), total glutathione peroxidase (GPx), glutathione reductase (GRed) and glutathione-S-transferases (GST) activities, and also lipid peroxidation levels (TBARS). Concerning the antioxidant responses studies in gills and liver, it was possible to observe alterations that were more evident in gills than in liver, after erythromycin exposure. Catalase quantification in gills showed a significant decrease of this enzyme’s activity in fish exposed to 0.05 and 0.08 µg/L. Our results showed that glutathione peroxidase activity was significantly increased only in gills of organisms exposed to 0.20 and 0.40 µg/L. No significant alterations were observed in GRed activity in gills and liver. Significant differences in GSTs activity were also found in gills after 0.1 - 0.8 µg/L ERY exposures. A significant increase in lipid peroxidation levels (shown by higher TBARS levels) occurred in gills, with the increase of erythromycin concentrations. It is thus possible to hypothesize that the higher level of TBARS constitute an indication of stress in cellular redox status (GPx and GST activities increased) mainly in gills, due to their physiological function of barrier and detoxification organ. The results obtained for liver tissue may be due to poor absorption of ERY, or to an overexpression of the enzymatic defense in gill tissue, thus preventing the establishment of an oxidative response in other tissues (such as liver).

MO051
Genotoxicity responses of environmental concentration of Oxytetracycline on fishes through micronucleus test and nuclear abnormalities
R.G. Botelho, Universidade de Sao Paulo / Ecotoxicology; C.A. Christofoleti, Fundacao Herminho Ometto / Environmental Mutagenesis; Y. Ansoar, Unesp / Bi; J.E. Correia, Universidade Estadual Paulista Julio de Mesquita Filho / Environmental Mutagenesis; v. tornisielo, Oxytetracycline (OTC) is one of the most commonly used antibiotic for the treatment of bacterial in fish farming in Brazil, and because of their intensive use, the potential harmful effects on aquatic animals is a great concern. In this study was evaluated the effect of environmental concentration of OTC on the genetic material of the juveniles of Tilapia (Oreochromis niloticus) erythrocytes by using occurrence of micronucleus (MN) and others erythrocitic nuclear abnormalities (ENAs). In a previous study at our laboratory the concentration 8,000 ng L⁻¹ was found in a Brazilian fish farming by Online-Liquid Chromatography coupled to Mass Spectrometry, and in the present study young of O. niloticus were exposed to this concentration, plus its half, 4,000 ng L⁻¹ and the double, 16,000 ng L⁻¹. The experimental design was composed of a repetition for each concentration and negative (NC) and positive controls (PC) with 6 fish in each, totaling 12 fish per treatment. At the final of the test (96 hours), blood from all fishes (n=12 per treatment) were collected by cardiac puncture dripped on microscopic glass slides and fixed with ethanol for 24 h. Then slides were stained with Leishman and Giemsa stain for 20 min. Three slides were prepared for each fish (n=5) of each concentration. From each slides a thousand erythrocyte cells (3,000 per fish) were analyzed under 1000x magnification through a light microscope and the images captured with a camera connected. ENAs was classified in three groups such as blebbed nuclei (BN), notched nuclei (NN), and lobed nuclei (LN). MNs were not registered in erythrocytes of O. niloticus after exposure to OTC and its respective NC and PC. Occurrence of ENAs were low and the most frequently ENA observed was NN. Significant differences were observed between PC and the lower concentration of OTC compared to NC (p≤ 0.05). Statistical differences were observed when compared the intermediate and the higher concentration of OTC and PC (p≤ 0.05). No significant differences were observed for the concentration of OTC and PC when compared to NC (p> 0.05) as well as for LN when compared the three concentrations of OTC and NC (p> 0.05). Statistical differences were observed when compared the intermediate and the higher concentrations of OTC in relation to PC (p< 0.05). In conclusion the environmental concentration of OTC and its half and double are not genotoxic to O. niloticus according to the MN test and ENAs.

MO052
Evaluation of Environmentally Relevant Concentrations of Oxytetracycline on Fishes using the Comet Assay
R.G. Botelho, Universidade de Sao Paulo / Ecotoxicology; J.E. Correia, Universidade Estadual Paulista Julio de Mesquita Filho / Environmental Mutagenesis; C.A. Christofoleti, Fundacao Herminho Ometto / Environmental Mutagenesis; Y. Ansoar, Unesp / Bi; v. tornisielo, Livestock production is one of the most expressive activity of Brazilian agribusiness. However, to increase the productivity and competitiveness, the use of drugs with therapeutic and prophylactic purposes is a common practice in Brazil. In Brazil, the fish farming, is one of the most important sector for the economy and which has been associated with negative impacts on aquatic environments due to the intensive use of these compounds. Oxytetracycline is one of the most commonly used antibiotic in Brazilian fish farming and thus, is very important to conduct toxicity test to verify how dangerous this is compound since a poor literature is available. Thus, the goal of this work was to evaluate the genotoxic response of rainbow trout (Oncorhynchus mykiss) to different concentrations of oxytetracycline (Oxytetracycline 2000 µg L⁻¹) using the comet assay. In a previous study, at our laboratory the concentration 8 µg L⁻¹ was found in a Brazilian fish farming by Online-Liquid Chromatography coupled to Mass Spectrometry, and in the present study young of O. niloticus were exposed to this concentration, plus its half (4 µg L⁻¹) and its double (16 µg L⁻¹). The experimental design was composed of a repetition for each concentration and negative and positive controls with 6 fish in each. After 96 hours exposure, blood from all fishes (n=12 per treatment) were collected by cardiac puncture, submitted to the comet assay in alkaline version (pH>13) under specific conditions for ADN denaturation, electrophoresis (running time: 20 min; Voltage: 39V and amperage 300 A) and then stained with GelRed. Comets were analyzed by visual methodology, considering the frequency of cells with or without damage, the score and the distribution of class. Kruskal-Wallis with p≤ 0.05 was used to compare the ADN damages between the groups treated with antibiotics and negative control. After the exposure time, ADN damages caused by environmental concentrations of oxytetracycline showed to be dose-dependent with the lower concentration presenting no damage and the intermediate and the
higher concentrations presenting higher damages.

MO053
Genotoxicity potential of environmental concentration of Florfenicol on fishes through micronucleus test and nuclear abnormalities

R.C. Carlyle Silva de Oliveira, Laboratory of Genetics and Toxicology / Departamento de Genética e Morfologia; D.S. Moura, Universidade de Brasília / Genética e Morfologia; R. Oliveira, Universidade de Brasília / Department of Genetics and Morphology; C.K. Grisolia, Universidad de Brasil / Department Genetics and Morphology

Pharmaceuticals are essential for the human and animal health. Antibiotics are among the most extensively used pharmaceuticals. Synthetic or natural drugs with the capacity to kill or inhibit the growth of microorganisms being mainly used in hospitals, agricultural fields, and livestock production facilities. Antibiotics can be administered to treat or prevent diseases as a prophylactic measure. Due to its high usage, relative persistence and constant input trough effluents the antibiotic have been detected in several aquatic environments. This study evaluated the genotoxic effects of sulfasalazine and nitrofurantoin using zebrafish embryos as model organisms. The toxicity tests were based in an extended version of the OECD protocol No. 236 - Fish Embryo Toxicity (FET) test. For each drug were prepared seven concentrations: 0; 1; 3.2; 10; 31.6; 100; 316 mg/L for sulfasalazine; and 0; 1; 2.2; 4.6; 10; 21.5; 46.4; 100 mg/L for nitrofurantoin. A total of sixteen organisms per concentration were evaluated using Daphnia magna toxicity tests. These antibiotics have been detected in high frequency in areas nearby large animal farms of Korea, but relevant ecotoxicological information is not available. The results of these tests confirmed the potential toxic effects of the antibiotic on zebrafish embryos. The data obtained in this study showed that a short-term exposure of zebrafish to sulfasalazine and nitrofurantoin can provoke alterations in the normal development of zebrafish only at high concentrations. Thus, adverse effects of the studied antibiotics are not expected to occur in natural ecosystems.

MO056
Chronical effects of cephradine and cefadroxil, two veterinary antibiotics, on aquatic organisms

B. Kim, Department of Environmental Health; K. Ji, Yongin University; J. Ryu, National Institute of Environmental Research / Risk Assessment Division; P. Kim, National Institute of Environmental Research; K. Choi, Seoul National University / School of Public Health

Veterinary medicines are frequently used to protect animal health, however these pharmaceuticals could enter the environment through direct application in aquaculture, runoff from manure-treated farmlands, or wash-off from topical treatments as nonpoint source pollutants. While many of these compounds have been recently detected in freshwater environment worldwide, their toxicological information is often limited. In the present study, the adverse effects of chronic exposure to cefadroxil and cephradine, cephalosporin antibiotics for animals, were evaluated using Daphnia magna and Danio rerio. These antibiotics have been detected in high frequency in areas nearby large animal farms of Korea, but relevant ecotoxicological information is not available. The chronic tests with D. magna (21 d) and early life stage toxicity tests with D. rerio (33 d) were conducted as outlined in the OECD test guideline 211 and 210, respectively. In the 21 d D. magna chronic toxicity tests of cephradine, the number of young per female (β = 0.375, p for trend = -0.004) and growth (β = 0.327, p for trend = 0.013) were significantly decreased in a dose-dependent manner. The no observed effect concentrations (NOECs) based on the endpoints of survival, reproduction, and growth in D. magna exposed to cephradine and cephradine were 100 mg/L, except growth NOEC for cephradine 50 mg/L. In juvenile zebrafish exposed to cephradine 33 d, the survival and growth endpoints were significantly affected at 10 mg/L. The effective concentrations of cephradine and cephradine were in a range between 10 and 100 mg/L, and therefore are several orders of magnitude greater than the levels potentially occurring in the ambient environment even including hotspots. Acknowledgement - This study was supported by the National Institute of Environmental Research in Korea.

MO057
Toxicity of the antibiotic sulfamethoxazole to Anabaena flos-aquae and Synechococcus leopoliensis, a comparison of cyanobacteria species sensitivity.

M. Baumann, Bavarian Environment Agency; K. Weiss; C. Polleichtner, Federal Environment Laboratory; D. Mallek, Federal Environment; D. Schudoma, Federal Environment

Synthetic drugs with the capacity to kill or inhibit the growth of microorganisms are considered as a major site for ROS generation. The objective of this study was to evaluate the embryotoxic effects of sulfasalazine and nitrofurantoin using zebrafish embryos as model organisms. The toxicity tests were based in an extended version of the OECD protocol No. 236 - Fish Embryo Toxicity (FET) test. For each drug were prepared seven concentrations: 0; 1; 3.2; 10; 31.6; 100; 316 mg/L for sulfasalazine; and 0; 1; 2.2; 4.6; 10; 21.5; 46.4; 100 mg/L for nitrofurantoin. A total of sixteen organisms per concentration were evaluated using Daphnia magna toxicity tests. The results of this study showed that a short-term exposure of zebrafish to sulfasalazine and nitrofurantoin can provoke alterations in the normal developmental of zebrafish only at high concentrations. Thus, adverse effects of the studied antibiotics are not expected to occur in natural ecosystems.
MO058 Effects of single veterinary and human pharmaceuticals and their mixtures on *Vibrio fischeri* V. Di Nica.

In this study toxicity effects of ten human and veterinary pharmaceuticals (PhCs) were tested by using the bioluminescent bacterium *Vibrio fischeri* as non target organisms. The compounds were investigated for their effects on growth, cell division and bioluminescence. The compounds were chosen according to their therapeutic application and concentration ranges in sewage waste cycling in marine and estuarine ecosystems. The effects were assessed by quantifying the number of living cells, cell viability, and the bioluminescence intensity. The results showed that some of the compounds, such as fluoxetine and oxazepam, had a significant effect on the growth and bioluminescence of *V. fischeri*. The study highlights the importance of assessing the effects of both veterinary and human pharmaceuticals on non-target organisms, as they can have significant impacts on aquatic ecosystems.

MO059 Effects of pharmaceuticals on the immunocompetence of the great pond snail *Lymnaea stagnalis* P. Rousseaux, P. Noury, Iristea Lyon; S. Betouille, Université de Reims; J. Garric, Iristea Lyon

Wildlife is exposed to multistresses (e.g. chemicals and microbiologicals). The immune system has a pivotal role in the health of organisms, particularly to clear pathogens and parasites. Many xenobiotics exert immunomodulatory features. Among them, pharmaceuticals are biologically active pollutants that may especially interact with internal defences of non-target species. Hence, we aimed at assessing the short-term immunotoxic effects of diverse pharmaceuticals on *L. stagnalis* under controlled laboratory conditions. Molecules were chosen according to their abundance in surface waters (e.g. psychotropic drug, anti-cancer drug) and/or their mean concentration of action (e.g. anti-inflammatory drug, immunosuppressive drug). Exposure concentrations scattered both, environmentally realistic and therapeutically-like concentrations. *Lymnaea stagnalis* has been chosen for several reasons. It is ecologically representative of lentic freshwater systems in Holartic regions of the planet. It is proposed at the OECD for full lifecycle toxicity tests and for reproactivity tests. As an invertebrate, it possesses an open and innate immune system. The study aimed at assessing the immunotoxic effects of pharmaceuticals on *L. stagnalis*. The results showed that some pharmaceuticals, such as fluoxetine, had a significant effect on the immune system of *L. stagnalis*. The study highlights the importance of assessing the effects of pharmaceuticals on non-target organisms, as they can have significant impacts on aquatic ecosystems.

MO060 Evaluation of oxazepam impact on the Radix balthica transcriptome during embryonic development J. MAZZITELLI, Centre Universitaire Jean-François Champollion, Albi, France / UCNRS, A. Puerti

The aquatic organisms are frequently exposed to a wide diversity of chemical compounds suspended in water or absorbed on substrates. These compounds, such as pharmaceuticals, are often found in the environment via polluted effluents, whether they are domestic or industrial. Most pharmaceuticals, notably psychotropic pharmaceuticals, are very persistent in the environment even after biological or abiotic treatment in waste water treatment plants (WWTP). The aim of this study was to assess the impact of oxazepam on the transcriptome of the clam *Radix balthica*. The study used a transcriptomic approach to identify the genes that were differentially expressed in response to oxazepam exposure. The results showed that oxazepam had a significant impact on the transcriptome of *R. balthica*. The study highlights the importance of assessing the effects of pharmaceuticals on non-target organisms, as they can have significant impacts on aquatic ecosystems.

MO061 Chronic toxicity of an antiepileptic on the clam *Venerupis philippinarum* A. Almeida, Departamento de Biologia; R. Freitas, Biology; V. Calisto, CESAM Department of Biology; V. Esteves, CESAM Universidade de Aveiro; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; E. Figueira, CESAM University of Aveiro

The presence of pharmaceutical drugs in the aquatic ecosystems is an emerging concern. The study aimed to assess the chronic toxicity of oxazepam on the clam *Venerupis philippinarum*. The study used a transcriptomic approach to identify the genes that were differentially expressed in response to oxazepam exposure. The results showed that oxazepam had a significant impact on the transcriptome of *V. philippinarum*. The study highlights the importance of assessing the effects of pharmaceuticals on non-target organisms, as they can have significant impacts on aquatic ecosystems.
two juveniles which could affect their feeding activity and growth. The third experiment performed with *Capitella sp* A juveniles and adults showed that fluoxetine enhanced preprotrogue individuals that may be beneficial for the population. Worms of *Capitella sp* A only showed delay or inhibition of copulation. This is the first experimental study that assessed effects of fluoxetine spiked sediments on development and reproduction of marine polychaetes. The observed fluoxetine adverse effects could have important ecological implications in natural populations due to the potential inhibition of reproductive processes. Therefore, this study can contribute to understand the possible responses of benthic invertebrates after exposure to this pharmaceutical in natural environments.

**MO063**

The Polychaete Diopatra neapolitana exposed to paracetamol: the use of the tissue regenerative capacity as a biomarker  
R. Freitas, Biology; D. Coelho, Universidade de Aveiro / Departamento de Biologia and CESAM; A. Pires, Universidade de Aveiro / Biologia; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; E. Figueira, B. Nunes, CESAM University of Aveiro; M. Novais, Polytechnic Institute of Leiria and University of Aveiro / ESTM GIRM PIL and Department of Biology CESAM; F. B. Espinoza, Universidad de la Habana; F. Beltrán, A. Espejo, Novais, Polytechnic Institute of Leiria and University of Aveiro / ESTM GIRM PIL and Department of Biology CESAM; E. Figueira, B. Nunes, CESAM University of Aveiro

Nowadays, there is an increasing concern about the large number of compounds present in the aquatic ecosystem, and the putative toxicological effects that may derive from their presence. Among these compounds one can find pharmaceuticals, which are continuously released into the wild by human activities. Although an increasing amount of data has been published on the occurrence of pharmaceuticals in the environment, a similar trend was not observed in terms of studies reporting and characterizing the potential deleterious effects of these compounds on non-target aquatic organisms, and information on their toxic effects in organisms other than human is still scarce. This is particularly true regarding the absence of data about physiological alterations undergone by polychaetes affected by this type of compounds. Essentially due to their life-history characteristics, as well as their capacity to colonize estuaries, these invertebrates are increasingly being used as sentinel species for anthropogenic (e.g. organic and inorganic contamination) and natural stresses (namely salinity alterations). Thus, in this study, the effects of the drug paracetamol were studied in the polychaete *Diopatra neapolitana*, using the tissue regenerative capacity as a biomarker. The results obtained revealed that both juveniles and adults affected by the higher tested concentrations exhibited significantly lower capacity to regenerate their body, in comparison with control organisms or even animals exposed to environmentally (lower) relevant concentrations. This study evidenced that paracetamol can significantly induce physiological alterations in *D. neapolitana*, resulting in overall diminished tissue regenerative capacity, which is of extreme significance for organisms with high ecological and economic relevance. Additionally, this study validates the use of *D. neapolitana* as a test organism in laboratory-based bioassays, but also as an adequate sentinel species to assess eco toxicological effects of pharmaceutical drugs. **Keywords:** Polychaetes, body regeneration, biomarker, pharmaceutical drugs

**MO064**

Antioxidant enzyme activities and lipid peroxidation in Daphnia magna exposed to an effluent spiked with pharmaceuticals and under single ozonation.  
A.O. Jiménez, Unidad de Toxicología Departamento de SanidadAnimal; S. Novais, Polytechnic Institute of Leiria and University of Aveiro / ESTM GIRM PIL and Department of Biology CESAM University of Aveiro; F. Beltrán, A. Espejo, Universidad de Extremadura / Departamento de Ingeniería Química y Química Física; C. Gravato, University de Porto / Laboratorio de Ecotoxicología; M. Lemos, Polytechnic Institute of Leiria and University of Aveiro / ESTM GIRM PIL and Department of Biology CESAM University of Aveiro

Application of Advanced oxidation processes used to remove pharmaceuticals from wastewater generates reactive oxygen species (ROS) that may reach superficial waters through discharges of effluents from sewage treatment plants. The main aim of this study was to clarify how increased levels of ROS produced after application of a single ozonation treatment of a real effluent would induce changes in the antioxidant enzymatic activities superoxide dismutase and catalase, that predominate responses against superoxide anion (O₂⁻) and hydrogen peroxide (H₂O₂) radicals respectively, and the level of oxidative damage (level of lipid peroxidation) in *D. magna*. Firstly, the wastewater was spiked with 9 selected pharmaceuticals (acetaminophen, antipyrine, caffeine, carbamazepine, diclofenac, hydrochlorothiazide, ketorolac, metoprolol and sulfamethoxazole at 200 µg L⁻¹ each) and then a single ozonation treatment was applied. Oxidative stress biomarkers were studied after an acute exposure period of 48 h to this treated effluent. The most marked change observed was an increase in the activity of SOD in daphnids that could be considered as a response to cope with increased levels of oxyradicals (O₂⁻) produced during the single ozonation treatment of the spiked effluent. **Acknowledgements:** Spanish CICYT, European Feder (Project CTQ2012/35789/C02/01), “Consejería de Empleo, Empresa e Innovación. Gobierno de Extremadura” and the Feder founds for the economic support.

**MO065**

Interaction between an emergent contaminant and variations in abiotic conditions: the role of salinity in the modulation of the ecotoxicological response of paracetamol  
B. Correia, CESAM University of Aveiro; R. Freitas, Biology; C.V. Silva; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; E. Figueira, B. Nunes, CESAM University of Aveiro

A large body of evidence has emerged, showing the contamination of aquatic environments by pharmaceuticals, due to their common usage and release at wastewater treatment plants and, consequently, into aquatic ecosystems. The biological activity of pharmaceuticals is an important parameter that can be modulated when evaluating its toxicological impact on aquatic organisms, since many species have similar pharmacological targets to those of humans. Paracetamol has analgesic and antipyretic properties being one of the most used and detected drugs in aquatic environments, with several studies reporting its toxicity towards a significant number of aquatic organisms. The ecotoxicological responses elicited by anthropogenic chemicals can be influenced by a number of abiotic variables, including those that may vary due to global changes. Among these, and with particular emphasis in estuarine environments, one can find salinity. In order to characterize the toxic response caused by paracetamol, the present work assessed the levels of the antioxidant defence system of the clam *Venerupis philippinarum*, including the activities of GSTs isoenzymes, catalase, superoxide dismutase, glutathione reductase; oxidative damage (lipoperoxidation); antioxidant balance (GSH/GSSG), under different salinity conditions. Animals were collected from the wild (Ria de Aveiro lagoon, Portugal), and acutely (96h) exposed to ecologically relevant paracetamol levels (0.05; 0.5; 5mg/L), under three distinct salinity values (14, 28 and 35 psu) simulating extreme and actual conditions already reported in the area. Significant differences were observed amongst parameters measured such as GSTs and GSH/GSSG, under salinities of 14 and 28 psu. It is possible to conclude that water salinity strongly influenced the response of the clams to different paracetamol concentrations, showing the importance of studying physiological traits under realistic test conditions, which are likely to vary in great extent as a result of climate change.

**MO066**

Effects of ibuprofen and carbamazepine on Na⁺/K⁺-ATPase system in Solea solea subjected to salinity changes.  
A. Gonzáles-Mira, Universitat de Valencia / Departamento Biología Funcional y Ambiental; F. Cordero, Universidad Complutense de Madrid; V. Budejovice / Faculty of Fisheries and Protection of Waters; R. Grabic, University of Zagreb / Faculty of Science

Solea solea (Solea solea) is a marine teleost that inhabits coastal and estuarine waters along the eastern Mediterranean area from the Adriatic Sea to the Black Sea. The knowledge of how temperature and salinity might interact to modulate the effect of some pharmaceuticals on the osmoregulation mechanisms in sole *Na⁺/K⁺-ATPase* alpha 1a1a and 1a1b subunits gene expression and *Na⁺/K⁺-ATPase* enzymatic activities were determined in different osmoregulatory tissues. Sole juveniles were acclimatized in four replicate tanks at two different temperatures, 15°C and 20°C, for a period of 60 days. After that period, they were administered an intraperitoneal injection (IP) of the non-steroidal anti-inflammatory drug (NSAID) ibuprofen (IB; 10 mg/Kg) and the anti-convulsing drug carbamazepine (CBZ; 1 mg/Kg). Non-injected fish, and those injected with the carrier (sunflower oil), acclimated at each of the two temperatures, were used for comparison. Ibuprofen showed an effect on the osmoregulatory mechanisms involved in the regulation of *Fiske* activity. In addition, the number of copies on both *Na⁺/K⁺-ATPase* alpha 1a1a and 1a1b subunits was higher at 20°C than at 15°C, which could be a direct response to temperature. This work was financed by the Ministry of Science and Innovation of Spain (Ref 458 CMT2010-16611) and by PROMETEO II/2014/085 (GV).

**MO067**

The effect of dexamethasone on the hepatic CYP1A2 and CYP3A4 response to the bacterial 16S rDNA 16611) and by PROMETEO II/2014/085 (GV).  
V. Búrka, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydroconos Vodnany Czech Republic; S. Sakalli, Faculty of Fisheries and Protection of Waters; O. Koba, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters; G. Zamaratskaia, Swedish University of Agricultural Sciences / Department of Food Science; G.T. Pham, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters; R. Grubic, University of
Effects of the pharmaceutical gemfibrozil on gilthead seabream S. MO069 reproductive capacity of zebrafish after chronic exposure to E2. However, the treatment with CAP reversed the reproductive function. The negative influence of synthetic hormone EE2 on the obtained by EE2 group also was significantly lower compared to the other groups, to EE2 group, before and after treatment with CAP. The number of eggs per female 0.05) in the gonadosomatic index (GI) between the control and E2 group comp

3 female fish were transferred to breeding tanks maintaining a ratio of 2 males for group : I) seven female fish were sacrificed for analyzing the gonads; II) 6 male and two test

evaluating the chronic toxicity of 17ß-estradiol (E2) and 17α-androstenedione (A) using

in waste and surface waters in concentrations of 7.2 - 3.0 ng/l and 4.3 - 2.6 ng/l, respectively. The overall CYP450 content. This hypothesis can be tested by determination of protein content of these CYP450s, which were not measured in this study. Thus, the eventual increase in the measured CYP450s remained invisible and did not lead to increase in total CYP450 content. This hypothesis can be tested by determination of protein content of individual CYP450 isoforms. DEX at the concentration 3000 ng/l did not alter any of investigated parameters suggesting biphasic regulation of CYP450 by DEX. In conclusion, DEX at the concentrations of 3, 30 and 300 ng/l can modify both total CYP450 content and CYP450 activity in rainbow trout. Keywords: Dexamethasone; CYP1A; CYP2E1-like protein; CYP1A. Acnowledgement - The study was supported by the MEYS of the CR - projects „CENAKVA“ (No.GZ.1.65.2.00/01.0023) and „CENAKVA II“ (No.LO206 and the NPU I program), by the Grant of the University of the BA GJU (No.087/2013/2E) and by the Czech Science Foundation (No.P503/11/1130).

Reversibility of reproductive efficiency in zebrafish exposed to 17ß-estradiol (E2) and 17α-androstenedione (A) after treatment with powered activated carbon (PAC) J. Silva, Sao Paulo State University - UNESP / Eng Ambiental; G.D. Alkimin, São Paulo State University - Campus of Sorocaba; S. Nakahira, Universidade Estadual Paulista UNESP; S. Daccache; C.C. Correa, Sao Paulo State University - Campus of Sorocaba; R. Fracacio, Unesp Endocrine disruptors, such as chemicals, have been found in wastewater, even after treatment, and they are known to affect the reproduction of local biota, indicating the necessity of effective treatments. Therefore, powered activated carbon (PAC) is a promising alternative for water treatment. The purpose of this study was evaluating the chronic toxicity of 17ß-estradiol (E2) and 17α-androstenediol (A) in zebrafish on reproductive phase, and to investigate the capacity of biological reversing of these organs after water treatment with PAC. 60 replicates with two-test organisms each, were exposed to chronic test. Among them, 23 replicates were submitted to 30 ng.L⁻¹ test-solution for each hormone (E2 and E2 group) during 21 days. The other replicates remained free of contamination and served as control group in this study. At the end of chronic period within each experimental group -I: seven female fish were sacrificed for analyzing the gonads; II) 6 male and 3 female fish were transferred to breeding tanks maintaining a ratio of 2 males for one female; III) other 23 fishes remained alive for the recovery test with a solution-treated with PAC until the 28th day of the experiment. Afterwards, the steps I and II were repeated. Kruskal-Wallis tests display significant differences (p < 0.05) in the gonadosomatic index (GI) between the control and E2 group compared to the PAC treated group. However, the recovery test with PAC reversed the reproductive capacity of zebrafish after chronic exposure to E2.

Effects of the pharmaceutical gemfibrozil on gilthead seabream Sparus aurata A. Barreto; L.G. Luis, CESAM & Department of Biology / Department of Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; K. Hylland, University of Oslo / Biosciences; S. Loureiro, Universidade de Aveiro / Biology; M. Oliveira, Laboratory of Ecotoxicology and Ecology

The presence of human pharmaceuticals in the aquatic environment is raising concern on their effects to the environment and human health. Blood lipid lowering agents are among the most commonly found drugs in the aquatic environment. Gemfibrozil, one of the most frequently detected pharmaceuticals from this class, has been considered a high priority pharmaceutical on the basis of its consumption, physicochemical properties, persistence and bioaccumulation. However, the study of its environmental fate and effects is quite scarce. This study was designed to evaluate the effects of this pharmaceutical to an estuarine/ marine fish, the gilthead seabream (Sparus aurata), after a 96-hour exposure. To that end behavioral and biochemical responses were assessed as endpoints. The consequences of short-term exposure of gilthead seabream to environmentally relevant concentrations of gemfibrozil was evaluated and the most sensitive biomarkers identified. Keywords: Toxicity, Biomarkers, Gemfibrozil, Sparus aurata. Acknowledgments: This research was supported through the COMPETE "Operational Competitiveness Programme and national funds through FCT – Foundation for Science and Technology, under the project "NANOAn – Effects of Gold Nanoparticles to Aquatic Organisms" (FCT PTD/MAE-EST/339/2012) (FCOMP-01-0124-FEDER-029435), “Pest-C/MA/LA0017/2013” and “Pic-C/CTM/LA0011/2013”; A. Barreto has a doctoral fellowship from FCT (SFRH/BD/97624/2013) and M. Oliveira a post-doc fellowship from FCT (SFRH/BPD/85107/2012) supported by the European Social Fund and national funds from the “Ministério da Educação e Ciência (POPH - QREN – Tipologia 4.1) of Portugal.

Neurotoxic potential evaluated in Corbicula fluminea exposed to human pharmaceuticals G.V. Aguiar-Martinez, Universidade de Cadiz / Chemical Physical; C. Andre, Environment Canada; P. Gagne, Emerging Methods, Environment Canada / Emerging Methods; A. DelValls, Universidade de Cadiz / Departamento de Quimica Fisica Facultad de Ciencias del Mar y Ambientales; L. Martin-Diaz, Universidade de Cadiz / Facultad de Ciencias del Mar y Ambientales

Adverse effects of pharmaceuticals on aquatic life have been demonstrated. However, for most drugs they are not well understood. This study aimed to evaluate neurotoxic potential on Corbicula fluminea after exposure to carbamazepine, novobiocin and tamofoxin. During 28 days water was spiked every 24 hours with 4 controls; 0: 0; 1; 10; 50 µg/L). Pharmaceuticals stocks were prepared in DMSO (0.001% v/v). Biomarkers studied in gonad tissues included Dopamine (DOP), Arachidonic Acid Cyclooxygenase activity (COX), Monooxidase Activity (MAO), Oxidase activity (MAO), Mitochondrial Electron Transport (MET), Total Lipids (TLP), Vitellogenin (VTG), and Energy Consumption (MET/TLP). Results showed a concentration-dependent relationship on DOP levels, MAO and TLP after exposure to drugs (p < 0.05). Neurotoxic effects included significant increase (p < 0.05) in DOP levels of clams exposed to carbamazepine and novobiocin, and to tamofoxin at 10 and 50 µg/L. The turnover of DOP was accompanied by increased catabolism, as evidenced by a significant increased MAO activity compared with controls (p < 0.05). MAO activity increased in clams exposed to novobiocin and tamofoxin at environmental concentrations, and to carbamazepine (10 and 50 µg/L). Drugs also induced significantly MET and COX activity compared with controls (p < 0.05). TLP increase significantly respect to controls in clams exposed to environmental concentrations of drugs (p < 0.01). Energy consumption did not differ between controls exposed to MAO and TLP. VTG decreased when increasing concentrations. Environmental concentrations of selected drugs have the potential to induce neurotoxicity. Selected biomarkers are suitable for environmental risk assessment of selected drugs in aquatic environment using C. fluminea as bioindicator species.

Responses of antioxidant defense system in liver and gill of rainbow trout (Oncorhynchus mykiss) chronically treated with dexamethasone V. Zlabek, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters LECHB; V. Burkina, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydrodences Vodnany Czech Republic; S. Sakalli, Faculty of Fisheries and Protection of Waters; A. Bofik, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydrodences Vodnany Czech Republic; G. Fedorova, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydrodences Vodnany Czech Republic; O. Koba, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters; G. Thay, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydrodences Vodnany Czech Republic; R. Grabic, University of South Bohemia / Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydrodences Vodnany Czech Republic; V. Wallas tests significance differences (p < 0.05) in the gonadosomatic index (GI) between the control and E2 group compared to the PAC treated group. However, the recovery test with PAC reversed the reproductive capacity of zebrafish after chronic exposure to E2.

Effects of the pharmaceutical gemfibrozil on gilthead seabream S. MO069
Measuring of antioxidants enzyme activities and damage from reactive oxygen species (ROS) become a useful tool in determination of harmful effects of organic pollution in aquatic ecology. Dexamethasone (DEX) is a potent synthetic member of the glucocorticoid class of steroid drug and has been found in effluents of sewage treatment plants at concentrations of 3-30 ng/L. In present study, the sublethal effects of DEX in juvenile rainbow trout were investigated, the response of antioxidant systems (superoxide dismutase, CAT, glutathione peroxidase and reductase, GPx and GR) and level of thiobarbituric acid reactive species were determined in liver and gill tissues after 21 and 42 days. This study showed that antioxidant defense system in fish was affected by DEX including environmentally relevant concentration after 21 days of exposure. The significant responses of fish to DEX exposure in SOD, CAT, and GPx activities displayed variant dependent on concentration of DEX. SOD activity was significantly induced by two highest DEX concentrations in liver after 21 days. CAT activity was affected by environmentally relevant concentration of DEX in liver and after 21 days. GPx activity in liver of fish exposed to 300 and 3000 ng/L for 21 days was significantly higher compared with control, while the activity of GPx was significantly lower in all DEX exposed groups after 42 days of exposure. Such result might suggest adaptive response of scavenger to ROS production during experimental period. Finally, lipid peroxidation level (measured as TBARS) was significantly induced by two highest DEX concentrations in liver after 42 days. The reason of oxidative stress could partly be due to detoxification process by cytochrome P450 system, which is potential source of ROS production (Burkina et al., 2014; poster at SETAC). In conclusions, results of the present study declare a potential harmful impact of regular DEX low concentrations, which may remain in aquatic micro-environments. This study raises concerns about environment. Therefore, it is important to obtain further knowledge about antioxidant systems in fish in relation to harmful organic pollutants.

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MO072
Morpurine disrupts innate immune system in zebrafish embryos: immunosuppressive effects and involvement of ABC transporters in lipopolysaccharide-induced inflammation

H. Mottaz, Eawag / UTOX; R. Schoenenberger, Eawag, Aquatic Research / UTOX; S. Fischer, Eawag / UTOX; A. Sepp, Friedrich Schiller University Jena / Institute for Environmental Toxicology; E. Köhler, Eawag / Department of Environmental Toxicology; K. Schirmer, Eawag / Environmental Toxicology; K. Ehrig, Eawag / UTOX Environmental Toxicology

Opioid drugs, such as morphine (MO), detected in waste and surface waters worldwide, may harm fish due to their semi-persistence and toxicity. Here, we studied the effects of MO on immune functions in fish. MO induces lipopolysaccharide (LPS) challenge assay was established, where waterborne LPS was added to promote inflammation and subsequent mortality, and time course of survival is monitored. Embryos were exposed to MO (concentrations confirmed by LC-MS/MS) from birth until LPS assay at 4 days post fertilization. Responses to LPS were bell-shaped, in that 100 ng/L-100 µg/L MO-exposed embryos exhibited significantly lower survival than MO-naïve embryos, while 1 µg/L MO-treated embryos did not differ from control (unexposed). RT-PCR analyses showed that MO above 10 µg/L caused progressive downregulation of various genes involved in inflammation, including MYD88, TRIF, TRAF6, p53, IL1β, IL6, IL8, CC-Chemokine and iNOS. Fish exposed to 1 µg/L accumulated 9.5 ng/g of MO after 4 days of exposure, a concentration comparable to that reported in blood of chronic drug users. These findings support the hypothesis that MO causes immunosuppressive effects of MO. The immunosuppression at higher MO concentrations apparently counteracts another influence that leads to increased inflammation at lower MO concentrations. This study revealed MO concentrations apparently counteracts another influence that leads to increased inflammation at lower MO concentrations. Thus, we suggest that MO-induced immunosuppression is an important tool in pathogenesis of MO-related disease.

MO073
Ecotoxicological evaluation of sedative-hypnotics drugs using zebrafish embryos as model organisms

L.Le Souza, Universidade de Brasilia / Departamento de Genética e Morfologia; C. Lisboa, Univeristy of Brasilia - Gtxs / Department of Genetics and Morphology; I.B. Ferraz, Universidade de Brasília Gtxs / Departamento de Genética e Morfologia; D.S. Moura, Universidade de Brasilia / Genética e Morfologia; R. Oliveira, University of Brasilia / Department of Genetics and Morphology; C.K. Grisolia, University of Brasilia / Department Genetics and Morphology

Zolpidem hemitartrate (Zol) and Bromazepam (Bro) are sedatives and hypnotics drugs that act on the central nervous system (CNS) decreasing its activity. Both, Zol and BRO are used to induce sleep-sedation and to treat anxiety. In spite of similar applications, Zol and Bro have different mode of action. Zol belongs to imidazopyridine class being a selective agonist of GABAB receptors, the major inhibitory neurotransmitter of CNS. On the other hand, Bro behaves to the class of benzodiazepines drugs, the most significant class of sedative-hypnotics being widely used in clinics. The aim of this study was to evaluate the toxic effects of two formulations of psychiatric drugs commonly used in Brazil, Zol formulation known as Noctiden (Biolab) and a Generic formulation of Bro (Biointeca). To do so, Developmental endpoints model organisms were used to perform a fish embryo toxicity evaluation. All Tests performed are based on an extended version of the OECD protocol No. 236 - Fish Embryo Toxicity (FET) test. For each drug were prepared seven concentrations: 0, 1.0; 1.9; 3.7; 7.2; 13.9; 26.8; 51.8 ng/mL for Zol; and 0.0; 0.4; 1.1; 3.5; 11.1; 35.0; 350 ng/mL for Bro. A total of sixteen organisms per concentration were divided in three 24-well microplates and exposed to 2 ml of test solutions. Developmental abnormalities, hatching, behaviour and mortality were assessed during 7 days. The 4d - LC50 for Zol were 38.2 and 59.2 mg/L, respectively. With the extension of the test time dependent toxicity trend was observed for both compounds especially for Zol (7d - LC50 = 17.1 mg/L). Effects on behaviour (loss of equilibrium, erratic swimming and paralysis) were observed for Zol in concentrations from 3.7 mg/L and for Bro in concentrations ten times higher (35 mg/L). Moerover, several malformations were induced including lack of pigmentation (eye and body), oedema, and tail deformities. For behavioural and embryotoxicoanalytical extension the addition of the test was essential to an accurate assessment of both drugs. Altogether, the effects induced by both drugs might indicate severe effects of these drugs in a chronic scenario of exposure. Little is known about the psychiatric drug in the environment. Additional tests designed considering the mode of action of sedatives and hypnotics drugs such neurological biomarkers (CNS gene expression), refined behaviour analysis and feeding test (food capture and prey avoidance) are recommended.

MO074
Effects of Gemfibrosil in Zebrafish embryo survival and larvae locomotory activity

J. Henriques, Universidade de Aveiro / Department of Biology; A.R. Almeida, University of Aveiro / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; I. Domingues, University of Aveiro / CESAM Department of Biology; M. Oliveira, Laboratory of Ecotoxicology and Ecology of Environmental Contaminants of Universidade de Aveiro / Department of Environmental Engineering

Gemfibrosil is a widely used in medicine to reduce blood concentrations of cholesterol and triacylglycerols. These class of pharmaceuticals are some of the most frequently reported in wastewaters and surface waters. Although most of these compounds have reduced and limited half-lifes, they may present the same exposure potential as persistent pollutants as these have a continuous input into the environment. Moreover, some studies have already demonstrated the negative effects of pharmaceuticals such as gemfibrosil on adult fish, however, survival and behavioural effects in fish larvae are lacking. In our work, we aimed to study the effects of Gemfibrosil in zebrafish embryo survival and larvae locomotory activity. Zebrafish embryos were exposed to both lethal and sub-lethal concentrations of the fibrate Gemfibrosil and lethality and sub-lethal endpoints were recorded daily for 96h. Sub-lethal concentrations where no apparent malformations occurred were used to test for behaviour analysis of 120-144 hpf larvae using a Zebrabox (Viewpoint®). Preliminary data shows that even in concentrations where no lethality occurred there seems to be an effect in fish locomotor behaviour.

MO075
Effects of the beta-blocker metoprolol and the NSAID diclofenac on the embryonic development and health of brown trout Salmo trutta f. fario

S. Schwarz, Animal Physiological Ecology; H. Schmiegl, Tübingen University / Animal Physiological Ecology; M. Scheurer, Water Technology Center TZW Karlsruhe; F. Sacher, DVGW-Technologiezentrum Wasser; H. Köhler, University of Tubingen / Animal Physiological Ecology; R. Triebskorn, University of Tübingen / Animal Physiological Ecology

The discharge of human and veterinary pharmaceuticals into surface waters via wastewater treatment plants is an increasing problem in central European countries, especially with respect to demographic changes. However, despite the frequent detection of various substances in aquatic ecosystems, knowledge on their effects in non-industrial species is largely lacking. Among the pharmaceuticals most frequently detected in European surface waters are some of the most frequently reported in wastewaters and surface waters. Pharmaceutical products are worldwide emerging contaminants due to a recreational input into the environment. Moreover, some studies have already demonstrated the negative effects of pharmaceuticals such as metoprolol and diclofenac on adult fish, however, survival and behavioural effects in fish larvae are lacking. In our work, we aimed to study the effects of Gemfibrosil in zebrafish embryo survival and larvae locomotory activity. Zebrafish embryos were exposed to both lethal and sub-lethal concentrations of the fibrate Gemfibrosil and lethality and sub-lethal endpoints were recorded daily for 96h. Sub-lethal concentrations where no apparent malformations occurred were used to test for behaviour analysis of 120-144 hpf larvae using a Zebrabox (Viewpoint®). Preliminary data shows that even in concentrations where no lethality occurred there seems to be an effect in fish locomotor behaviour.
diolcefenac already exist, the ecotoxicological potential of environmentally relevant concentrations is still discussed. In the case of metoprolol only very few studies are available. Our study investigated the effects of the two substances on the health status and on the embryonic development of brown trout, Salmo trutta f. fario, a species of high local ecological relevance for central Europe. The applied concentrations were 0.1, 0.5, 1, 10, and 100 µg/L for diclofenac, and 0.1, 1, 10, 100, and 1000 µg/L for metoprolol including several concentrations in ranges that have been reported for German surface waters. Juvenile fish were exposed for a time period of 27 days. Besides biometric measurements and mortality, the level of the stress protein Hsp70 was measured as a general marker of proteotoxicity. Furthermore, the degree of lipid peroxidation, which is supposed to be reduced by NSAIDS, and the histological status of liver, kidney, gill, and heart (only for metoprolol) as an indicator for overall health, was investigated. The exposure of trout eggs embrions was based on OECD 212. Recorded endpoints included mortality, time till eye development, time to hatch, number of hatched larvae, and number of healthy larvae at the end of the test. Overall, our study aims at providing further information on the effects of environmentally relevant concentrations of two frequently used pharmaceuticals in a species of high ecological relevance in Germany and adjacent countries.

MO076

The ecotoxicological effects of 4 common human anti-inflammatory agents on the unicellular green algae Chlamydomonas reinhardtii

M. Pino, San Jorge University / Facultad ciencias de la salud; S. Muniz, D. Chinarro, San Jorge University; E. Navarro, CSIC - Spanish National Research Council / Biodiversity conservation & Ecosystems Restoration

Pharmaceutical compounds such as non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics are used extensively worldwide and their consumption, predominantly in developed countries, is assumed to be higher than several hundred tons per year (Daughton and Ternes, 1999). Approximately 70% of administered drugs are estimated to be released into the environment (Jacobson and Berglund, 1988), mainly as complex mixtures via both untreated and treated sewage (Ternes et al., 2004; Yu et al., 2011). That would explain the increasing evidence of pharmaceutical ubiquity in the environment. Although the global impact of pharmaceuticals in ecosystems still remains unclear, in vitro and in vivo assays have demonstrated their ecotoxicity effects on many non-target organisms. Thus, the aim of this study was to measure the acute ecotoxicity of four NSAIDs with a widespread presence in the environment (ibuprofen, diclofenac, paracetamol and salicylic acid) by using for the first time the algae Chlamydomonas reinhardtii as aquatic model organism. Our results indicate that all of the studied drugs are likely to cause toxic effects on the photosynthetic yield of Chlamydomonas reinhardtii (EC50 in the range of 660 to 3866 mg/L). The salicylic acid was the most toxic followed by ibuprofen, diclofenac and paracetamol. However, and based on the concentrations found in natural waters, the four NSAIDs are not expected to represent an acute risk, for this aquatic biota.

MO077

Anthropogenic gadolinium: environmental concentrations in Lorraine region (France) and effects on living organisms

E. Perrat; M. Parant, Universite de Lorraine; C. ROSIN, ANSES / Member of the Working Group; C. Cossu-Leguille, Laboratory LIEBE - CNRS UMR 7146 - Université de Lorraine

Gadolinium-based contrast agents (Gd-CA) are often used in Magnetic Resonance Imaging (MRI). After intravenous injection (from 160 to 240 mL/Mg), these compounds are released by urinary excretion for several weeks (Kummerer et al., 2004; Yu et al., 2011). That would explain the increasing evidence of pharmaceutical ubiquity in the environment. Although the global impact of pharmaceuticals in ecosystems still remains unclear, in vitro and in vivo assays have demonstrated their ecotoxicity effects on many non-target organisms. Thus, the aim of this study was to measure the acute ecotoxicity of four NSAIDs with a widespread presence in the environment (ibuprofen, diclofenac, paracetamol and salicylic acid) by using for the first time the algae Chlamydomonas reinhardtii as aquatic model organism. Our results indicate that all of the studied drugs are likely to cause toxic effects on the photosynthetic yield of Chlamydomonas reinhardtii (EC50 in the range of 660 to 3866 mg/L). The salicylic acid was the most toxic followed by ibuprofen, diclofenac and paracetamol. However, and based on the concentrations found in natural waters, the four NSAIDs are not expected to represent an acute risk, for this aquatic biota.

MO079

Bioaccumulation and trophodynamics of sertraline and fluoxetine in laboratory fertilized three-level aquatic chains

A. Kiefer, M. Berglund, Lund University / Dept of Biology; M. Bostrom, Lund University / Biology; J. Jonsson, Lund University / Center for Analysis and Synthesis

To test the influence of food on bioaccumulation of pharmaceuticals we exposed two-three level aquatic food chains to the selective serotonin reuptake inhibitors (SSRIs) sertraline and fluoxetine in a laboratory set-up. The constructed food chains shared the two lower trophic levels (detrital maple leaves, Acer platanoides fed on by the detritovorous isopod Asellus aquaticus), but differed in the consumers (the water beetle Notonecta glauca, and the nine-spined stickleback Pungitius pungitius, both feeding on A aquaticus). Neither of the SSRIs bioaccumulated in the food chain, concentrations showed a decreasing trend from detritus to isopods to predators. Bioaccumulation factors (BAFs) calculated as concentration in organism divided with concentration in water were for sertraline: 2200±1300 for detritus, 360±230 for isopods, 26±10 for N glauca and 49±37 for P pungitius, all on a wet weight basis. The corresponding BAFs for fluoxetine were: 1300±660, 11±50, 11±4, and 41±34. BAFs for the organisms feeding on contaminated food were not different to bioconcentration factors (BCFs) measured in a separate experiment with water exposure only. Thus, food or trophic transfer did not have a major influence on the accumulation of the SSRIs in this study.

MO080

The uptake and elimination of selected pharmaceuticals in the aquatic invertebrate, Gammarus pulex

T.H. Miller, Kings College London / Analytical and Environmental Sciences; R. Brown, ENTPE / ENTPE; S. Muniz, D. Chinarro, San Jorge University; E. Navarro, CSIC - Spanish National Research Council / Biodiversity conservation & Ecosystems Restoration

A. Karplus, Kings College London / Division of Diabetes and Nutritional Sciences; L. Barron, Kings College London / Analytical and Environmental Science

Through the contamination of surface water, aquatic biota are exposed to a varying number of contaminants such as the class of pharmaceutical and personal care products (PPCPs). Sources of contamination are a result of anthropogenic activities (agriculture, landfill sites, health and veterinary services). The exposure of these organisms to pharmaceuticals has been shown to have varying toxic effects in reproduction and also affect behaviour, yet data on their toxicokinetics are lacking. Therefore, the understanding uptake and elimination of pharmaceuticals in aquatic life is essential to identifying compounds that may pose significant risk through the accumulation via water or diet. Herein, we present a method for the determination of toxicokinetics of several pharmaceuticals in the aquatic invertebrate, Gammarus pulex. Field-collected Gammarids were acclimatised in the laboratory for use in the exposure experiments. G. pulex were exposed individually to environmentally relevant concentrations of eight radiolabelled pharmaceuticals (14C or 1H) including propranolol, diclofenac, metoprolol, ibuprofen, imipramine, terbutaline, ratalidine and fluoxetine. The exposure organisms were bioaccumulated by a 48 h depuration period. Water and organism concentrations were quantified by liquid scintillation counting. The uptake and elimination rates were modelled simultaneously as specified in the OECD 305 guidelines and a kinetic bioconcentration factor (BCF) estimated. Estimated BCFs remained below 200 for several compounds with varying hydrophobicity, indicating little bioconcentration-like effects occurred via water exposure. BCF studies in this organism enables a much simpler and less time consuming analysis than traditional fish bioconcentration studies. Such studies may provide insight in the future as to whether a compound has the potential to accumulate through biomagnification. This study contributes to the understanding of pharmaceutical toxicokinetics in G. pulex invertebrates and the method used may highlight humans when used for MRI (Amet & Deray, 2012; Galera et al., 2010). Keywords: Bioaccumulation; Ecotoxicity; Gadolinium-based contrast agents; Aquatic freshwater organisms

MO081
A novel approach for reducing emissions of pharmaceuticals to the environment


There is increasing interest over the impacts of pharmaceuticals on the natural environment. Inputs from inappropriate disposal of unused medicines and emissions from manufacturing plants are thought to be important pathways for pharmaceuticals to enter the environment. A recent survey that we conducted on what people do with unused medicines found that 30% of people questioned disposed of surplus medicines inappropriately and only 17% were aware of schemes that enable medicines to be returned to the pharmacy for transportation to, and disposal in, a high temperature incinerator. By better managing unnecessary emissions, levels of pharmaceuticals in surface waters, groundwaters and soils could be reduced. Pyropyre is a new small scale treatment unit that has the potential to destroy pharmaceutically contaminated wastes on site by processes of pyrolysis and gasification. While the system operates at around 550 °C a temperature that should be sufficient to destroy many organic molecules, its efficacy for treatment of pharmaceuticals is unknown. This study therefore investigated the suitability of the Pyropyre system for 17 of the most thermally stable pharmaceuticals that are currently in use. The system was applied to three waste scenarios containing the test substances: sharp waste bins containing needles and syringe bodies; pharmaceuticals to take back containing packaging and blister packs; and manufacturing waste to simulate waste from a production line. Samples of the effluent, settleable solids and gas emissions from the system were obtained and analysed for parent compounds and transformation products using a combination of LC-MS/MS and LC-FT-MS. Results suggest that Pyropyre is an effective treatment process with in-excess of 99% of the applied pharmaceuticals destroyed for ten of the 17 and an average of 94% destroyed for the other seven. None of the ten active metabolites were detected in significant quantities (less than 0.6% of parent equivalent). Using the destruction level achieved by the PyroPure system the contribution to existing levels of pharmaceutical contamination in wastewater of a single PyroPure operating at full capacity was assessed. The outputs from the model suggests that future implementation of the PyroPure system on a large scale scheme could help to reduce the levels of pharmaceuticals in the environment whilst also reducing the economic and environmental costs of current waste management practices.

MO082
Excipients from galenical pharmaceutical production in wastewater: overview and environmental risk assessment

K. Wirz MA, F.Hoffmann-La Roche Ltd / Site SHE; M. Studer, F.Hoffmann-La Roche AG / Site SHE; J. Strub, F.Hoffmann-La Roche Ltd / Roche Group Safety Health Environmental Protection

In galenical production, many excipients are processed for different purposes, in addition to the active pharmaceutical ingredients (APIs). Compared to the APIs, excipients are little investigated regarding their environmental impact and fate, even though some of them are used in large quantities. To investigate potential effects and risks to aquatic biota and the environment arising from excipients during galenical production, the chemical, physical and environmentally relevant properties, in particular biodegradability and toxicity towards aquatic biota, of different excipients were collated and a predicted no effect concentration (PNEC) was calculated where possible. Overall, 184 excipients were literature-searched for properties. Due to the fact that the database is insufficient, only half of the excipients allowed an aquatic PNEC calculation. Based on these parameters, for each of the 42 excipients currently used in galenical productions at Roche Basle and Roche Kaiseraugst, both in Switzerland, a predicted environmental concentration (PEC) was derived from maximum daily loss of the single excipients to wastewater, derived by mass balance. Then, an environmental risk assessment comparing PEC and PNEC was conducted for the respective wastewater treatment plant (WWTP) and the receiving surface water, the River Rhine for both plants. Additionally, to simulate a theoretical worst case scenario, certain galenical processes where given excipients are used in the highest amounts were assumed to take place in parallel on the same day, resulting in increased excipient losses to wastewater. The PEC/PNEC risk characterisation ratios for the excipients currently used at Roche are well below 1 throughout. Together with the fact that based on biodegradability data many will be removed in the WWTP, this indicates that the excipients currently used do not present a risk to the environment. However, as the database is incomplete, environmental risks from excipients in galenical production in general should not be automatically considered to be negligible. Further work on excipients, specifically their biodegradability and effects on the environment, is needed to assemble a more solid and transparent database, in order to preclude adverse effects in WWTPs and receiving waters from galenical production.

MO083
Toxicity of seaweeds from the Persian Gulf

M. Yousefzadi, Marine Biology; S. Mashjoor, Hormozgan University; A. Naji

Conclusion: The results obtained in this study showed that Ethyl acetate and methanol extracts of marine Macro algae U. flexuosa, P. antillarum and P. boergesenii. Species used in this paper has a great potential for future uses, properties, in addition to cytotoxic activities. These data indicate the possibility that seaweeds and its constituents may be applied as drug (antibacterial and anticancer agent) for human administration.

Evolutionary, multigenerational and epigenetic effects of pollutants: scientific support to long-term ERA (P)

MO084
Genetic variation of Lymnaea stagnalis sensitivity to copper sulfate and its implication for toxicity testing

M. Contat, INRA / E Development and Ecosystem Health UMR INRA Agropolis Ouest; J. Cöke, INRA; A. Boucard, INRA / Ecology and Ecosystem Health UMR INRA Agropolis Ouest; Y. Pronost, INRA / Experimental Unit of Aquatic Ecology and Ectotoxicology; M. Cöke, INRA; A. Besnard, INRA / UMR ESE 0985; F. Pigué, ISAE; T. Caquet, INRA / UMR ESE

The use of monospecific standardized tests to assess the ecological risk of chemicals implicitly relies on the assumption that intraspecific variation in sensitivity is negligible in this context. First, this is a very strong hypothesis, and current research provides overwhelming evidences of significant variation among conspecific lineages in their ability to face environmental change and stress. Second, population response to stress has both a plastic and a genetic component, the latter, if additive, forming the basis for adaptive evolution. This means that population divergence in chemical sensitivity may be genetically fixed, as the result of exposure history (genetic adaptation). In this study, we investigated genetic variation in pesticide sensitivity of a non-target species, the freshwater snail Lymnaea stagnalis. We focused on a set of 64 lineages stemming from eight natural populations or strains found to be genetically differentiated at neutral markers and life history traits (for natural populations, see Boucard et al. 2014, PLoS ONE 9; e106670). Snails representative of at least the third generation born in the laboratory since initial sampling were exposed to copper sulfate at a concentration determined as global 96h LC50 (pilot experiment, all lines mixed up). Survival over time was modeled using a lognormal distribution (lowest AIC) and regressed on the model chemical treatment x population + body size (R function survreg). All effects were highly significant, except that of treatment by population interaction (p = 0.095). However, copper sensitivity varied significantly among populations (Tukey post-hoc test; 96h survival: 50 to 90%, mean = 69.7%, SD = 12.6%), as did the daily death rate (8 to 25%; mean = 16%, SD = 5.2%). These findings clearly show that extrapolating results from “single-line” toxicity tests to the species level can lead to strongly biased conclusions. One possibility of improvement might be to explicitly include conspecific lineages of various genetic origins in standardized testing procedures.

MO085
Facing multiple stressors: Genetic variability and structure of a model freshwater species

P. Isnoten, Helmholtz Centre for Environmental Research GmbH - UFZ / Effect Directed Analysis; S. Michalski, Helmholtz Centre for Environmental Research UFZ / Community Ecology; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis; H. Norf, Helmholtz Centre for Environmental Research UFZ / River Ecology Aquatic Ecosystems Analysis and Management

Multiple stressors including micropollutants are currently receiving growing interest due to increasing concerns about ecosystem health. Many of such multiple stressors occurring in the environment can exert strong effects on ecological communities as a consequence of both synergistic and antagonistic effects on organisms. In addition, they can also interact and induce unexpected consequences thereby changing natural dynamics. Field studies addressing ecological effects of multiple stressors, however, are still scarce. Hence we used the amphipod Gammarus pulex as a model to test the hypothesis that changes in the intraspecific structure of natural populations correlate with land-use driven factors such as the concentration of micropollutants. The hypothesis was tested by collecting seasonal samples of G. pulex from two agricultural and one natural stream in the Harz Mountains, Germany. In total 24 specimens per site and date were analysed using 18 microsatellite loci. Even if we found similar seasonal dynamics in the genetic structures of amphipod populations inhabiting natural and agricultural streams, we observed generally higher genetic diversity in agricultural than in natural streams. Our results emphasize the need for high temporal resolution when addressing stressor effects on the intraspecific structure of ecological populations.

MO086
Epigenetic memory of environmental stressors in Daphnia magna

C. Goncalves Athanasio, University of Birmingham / Biosciences; L. Mirbahai, The University of Birmingham / School of Biosciences; S. Parameswaran, C.
Gopalakrishnan, Genotypic Technology Pvt Ltd; M.R. Viant, University of Birmingham / School of Biosciences; J. Chipman, The University of Birmingham / School of Biosciences

Cytosine methylation plays an important role in regulation and stability of gene expression, providing a cellular memory especially for long-term silencing of genes. It is well known that several pollutants can modify epigenetic marks which can lead to changes in gene expression and potential to cause adversity. The epigenetic changes can be maintained throughout lifetime, and potentially beyond into subsequent generations, emphasizing the concept of epigenetic memory. It is proposed that class-specific alterations to the normal pattern of DNA methylation may occur due to chemicals and other stressors, offering a unique opportunity to provide a lifetime-history of an organism’s prior exposure to factors influencing the epigenome. Thus, the use of epigenetic profiles may provide a system to reflect early- or long-term exposures to stressors, and have utility as a novel tool for environmental monitoring. To explore these hypotheses, Daphnia magna was exposed for 14 days to sub-lethal concentrations of 5-acetyladenine (a demethylating agent; 3.7mg/L), arsenic (a carcinogen known to modulate the epigome; 100μg/L) and hypoxia (an environmental stressor; <2mg/L). Life-history traits along with hemoglobin quantification were used to identify the effects of the stressors on the organisms. Whole-genome bisulphite sequencing (WGBS) was used to identify modified DNA methylation profiles. The sustained exposure of adult daphnids to either 5-acetyladenine or hypoxia caused a decrease in the growth rate of the subsequently obtained third brood compared with controls. Daphnia exposed to hypoxia also presented a 2-fold increase in haemoglobin concentration compared with controls. This was correlated with the maternal food exposure. In contrast to the hypoxia group, arsine (arsenic) induced the expression of the maternal gene. On the other hand, derived brood was observed following exposure of the parent animals (or the brood themselves) to arsenic. WGBS enabled the characterisation of the profile of methylated cytosines across the genome of the exposed adult daphnia. Global methylation level was approximately 0.4 % and this global level was not significantly altered in response to any of the above treatments. The sequencing data are now being interpreted to determine if the epigenomic effects of gene-specific epigenetic modifications occur and, if so, to determine the persistence of such changes. The study thus tests if stress-specific DNA methylation profiles might present an opportunity to be used for identification of history of adverse exposures.

MO087
Multigenerational effects of Daphnia magna exposed to polluted field water N. Chatterjee, S. Choi, s. Lee, University of Seoul; J. Choi, School of Environmental Engineering Graduate School of Energy and Environmental system Engineering

In agreement with the thoughts that multigenerational and/or micro-evolutionary effects of chemicals should be incorporated in risk assessment, we, herein, report a comprehensive study with different phenotypic as well as physiological responses of Daphnia magna cultured in field water which was collected from surface stream water with industrial as well as natural contaminants. Phenotypical and physiological responses of Daphnia cultured in polluted field water were compared with those cultured in standardized Daphnia media (i.e. M4 media). On one hand, increase in body size, spine length, reproduction rate, hemoglobin formation were observed, on the other hand, slower movement and decrease in heart rate were evident in field water exposed animals. The possible correlation of molecular mechanism and phenotypical phenomena is currently ongoing in our lab. Taken together, our results present a paradigm not only of multigenerational effects of environmental pollutants but also add new dimensions of observation about complex mixture stressors with real field water. Acknowledgement: This work was supported by the grant from the Korea Ministry of Environment as "Environmental Health R&D Program" (2014001370004)

MO088
Multi-generational effects of nanoparticles on Caenorhabditis elegans S. Kim; J. Moon, Konkuk University; Y. An, Konkuk University / Department of Environmental Science

The nematode Caenorhabditis elegans is a key species used in nano-toxicity assessment. They normally lay embryos that hatch outside the body, but embryos are retained and hatched inside the parental body, a phenotype called a "bag of worms" (BOW). On nanoparticle exposure condition, this phenomenon can lead to changes in gene expression and potential to cause adversity. The epigenetic changes can be maintained throughout lifetime, and potentially beyond into subsequent generations, emphasizing the concept of epigenetic memory. It is proposed that class-specific alterations to the normal pattern of DNA methylation may occur due to chemicals and other stressors, offering a unique opportunity to provide a lifetime-history of an organism’s prior exposure to factors influencing the epigenome. Thus, the use of epigenetic profiles may provide a system to reflect early- or long-term exposures to stressors, and have utility as a novel tool for environmental monitoring. To explore these hypotheses, Daphnia magna was exposed for 14 days to sub-lethal concentrations of 5-acetyladenine (a demethylating agent; 3.7mg/L), arsenic (a carcinogen known to modulate the epigome; 100μg/L) and hypoxia (an environmental stressor; <2mg/L). Life-history traits along with hemoglobin quantification were used to identify the effects of the stressors on the organisms. Whole-genome bisulphite sequencing (WGBS) was used to identify modified DNA methylation profiles. The sustained exposure of adult daphnids to either 5-acetyladenine or hypoxia caused a decrease in the growth rate of the subsequently obtained third brood compared with controls. Daphnia exposed to hypoxia also presented a 2-fold increase in haemoglobin concentration compared with controls. This was correlated with the maternal food exposure. In contrast to the hypoxia group, arsine (arsenic) induced the expression of the maternal gene. On the other hand, derived brood was observed following exposure of the parent animals (or the brood themselves) to arsenic. WGBS enabled the characterisation of the profile of methylated cytosines across the genome of the exposed adult daphnia. Global methylation level was approximately 0.4 % and this global level was not significantly altered in response to any of the above treatments. The sequencing data are now being interpreted to determine if the epigenomic effects of gene-specific epigenetic modifications occur and, if so, to determine the persistence of such changes. The study thus tests if stress-specific DNA methylation profiles might present an opportunity to be used for identification of history of adverse exposures.

MO089
Exposure of zebrafish to morphine during first day of development results in modulation of innate immune system responses at later stages H. Mottag, Eawag / UTOX; R. Schoenenerberger, Eawag, Aquatic Research / UTOX; R.I. Eggen, Eawag / Department of Environmental Toxicology; K. Schirmer, Eawag / Environmental Toxicology. K.-J. Groh, Eawag / UTOX Environmental Toxicology

Opioid drugs, such as morphine (MO), are detected at concentrations up to 1 μL/L in waste and surface waters worldwide, as a consequence of both licit and illicit use. These compounds may pose a risk to aquatic organisms due to their semi-persistence and potency, in particular to fish due to conservation of molecular drug targets across vertebrates. In mammals, MO is known to influence not only behavior but also the immune system and epigenetic regulation. We have recently observed that continuous exposure to MO from 1 hour post fertilization (hpf) to 4 days post fertilization (dpf) modulates the innate immune system responses in zebrafish (Danio rerio) embryos, as assessed by bacterial lipopolysaccharide (LPS) challenge assay. Excavation of LPS-induced inflammation and immunosuppressive effects were seen at lower and higher MO concentrations respectively. These could be explained by the observed downregulation of several relevant genes, including MYD88, TRIF, TRAF6, p38, NFκB2, IL1β, IL6, IL8, CC-Chemokine and iNOS, as well as ABC transporters, assessed by RT-PCR. In this study, we set out to investigate whether shorter exposure to MO during early life would be sufficient to evoke similar effects at later stages. The embryos were exposed to MO from 1 hpf to 26 hpf, followed by washing and raising in clean water until gene expression analyses or LPS assay at 4 dpf. We observed that short-term early life exposure to MO resulted in gene expression changes similar to those seen after continuous exposure. Moreover, modulation of responses to LPS followed similar patterns. Previous studies reported that MO is cleared out from adult zebrafish within 1 day after waterborne exposure, indicating that the effects we observed may be due to persistent effects of MO on gene expression induced during short-term early life exposure. Ongoing work focuses on MO uptake and depuration kinetics in zebrafish embryos. If the depuration of MO by 4 dpf where gene expression is assessed will be confirmed, the potential epigenetic effects of MO will be investigated using bisulfite sequencing to assess the promoter methylation status of relevant genes. This work demonstrated that short-term exposure to MO during early life potently modulates innate immune system responses at later stages, raising concerns about potential adverse effects on the fish immune system following short-term exposure of sensitive life stages to environmentally present opioid drugs.

MO090
DNA alterations and effects on growth and reproduction in Daphnia magna during chronic exposure to gamma radiation over three successive generations F. Parisot, IRSNPRP-ENVLECO / LECO; J. Bourdin, Géochimie et Ecotoxicologie des Métaux dans les systèmes Aquatiques; C. Adam-Guillemard, IRSN, F. Alonzo, Eawag, Aquatic Research / UTOX

Anthropogenic activities related to the nuclear industry contribute to continuous discharges of radionuclides into terrestrial and aquatic ecosystems. Over the past decade, the potential risk of ionizing radiation for natural biota has become a growing public, regulatory and scientific concern. Until recently, ecological risk assessments of ionizing radiation have mainly focused on chronic effects measured over exposure durations, which are not really relevant of realistic situations. In this paper, effects of chronic radiation on Daphnia magna are studied. The effects were compared with controls to also assess the potential for ionizing radiation to affect the immune system and epigenetic regulation. We have recently observed that continuous exposure to γ radiation from 1 hpf to 26 hpf, followed by washing and raising in clean water until gene expression analyses or LPS assay at 4 dpf. We observed that short-term early life exposure to γ radiation resulted in gene expression changes similar to those seen after continuous exposure. Moreover, modulation of responses to LPS followed similar patterns. Previous studies reported that MO is cleared out from adult zebrafish within 1 day after waterborne exposure, indicating that the effects we observed may be due to persistent effects of MO on gene expression induced during short-term early life exposure. Ongoing work focuses on MO uptake and depuration kinetics in zebrafish embryos. If the depuration of MO by 4 dpf where gene expression is assessed will be confirmed, the potential epigenetic effects of MO will be investigated using bisulfite sequencing to assess the promoter methylation status of relevant genes. This work demonstrated that short-term exposure to MO during early life potently modulates innate immune system responses at later stages, raising concerns about potential adverse effects on the fish immune system following short-term exposure of sensitive life stages to environmentally present opioid drugs.
describe how an accumulation and transmission of DNA alterations can explain changes in growth and reproduction effects over successive generations and explore the metabolic mode of action associated with gamma radioactivity.

MO091 Effects of an environmental relevant concentration of carbendazim on twelve generations of Daphnia magna
A.R. Silva, University of Aveiro / Deptof Biology CESAM; D.N. Nunes Cardoso, CESAM, University of Aveiro / Department of Biology and CESAM Centre for Environmental and Marine Studies; A.S. Cruz, CESAM & University of Aveiro / Biology; S. Mendo, University of Aveiro / Department of Biology CESAM; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; S. Lourenço, Universidade de Aveiro / Department of Biology and CESAM.

In the environment, organisms can be exposed to chemicals continuously or for a long-term. Due to runoff from agriculture fields, pesticides may appear in surface waters and contribute to this long-term exposure. Carbendazim is one of the pesticides that has been detected at concentrations near 5 µg/L, as it is a fungicide widely used in agriculture in plant production products, including fruit trees, cereals, vegetables, etc. Therefore, testing effects throughout several generations of organisms can be a good epigenetic approach to evaluate these long-term exposures. Daphnids can thus be good models due to their parthenogenetic reproduction, where there is no recombination, and consequently no interference of genetic variability between organisms. Considering this, Daphnia magna was exposed to an environmental relevant concentration of carbendazim (5 µg/L for two femine generations of male fish is a widespread phenomenon. The Grand River watershed of the present study was to investigate the effect of carbendazim on successive generations of D. magna, considering the effects of the contaminant in the offspring, when parents were pre-exposed to the pesticide. For that, currently used ecotoxicity tests (e.g. immobilisation, feeding inhibition or reproduction tests) and a genotoxicity test (the comet assay) were carried out throughout generations exposed to carbendazim. The obtained results indicated that there were differences on carbendazim sensitivity upon exposure of daphnids born in clean medium and daphnids born in medium already containing carbendazim, at all endpoints tested (except in the immobilisation data). In addition, DNA damage was higher in generations of daphnids born in carbendazim (compared with the ones born in clean medium) and DNA damage appeared to be transferred from mother to the offspring along the generations, but it also increased from generation to generation.

MO092 Blame your parents! Parental effect impacts progeny response to municipal wastewater effluent exposure.
M. Fuzzen, University of Waterloo / Biology; L. Bragg, University of Waterloo / Department of Biology; G. Tetreault, University of Waterloo / National Water Research Institute; M.E. McMaster, Environment Canada / National Water Research Institute; M.R. Servos, University of Waterloo / Department of Biology. Over 30 fish species have been reported to show an intersex condition (ova-testes) after exposure to municipal wastewater effluent (MWWE), suggesting that the female generation of male fish is a widespread phenomenon. The Grand River watershed in southern Ontario, Canada is home to one million people serviced by 30 municipal wastewater treatment plants (MWWTPs). The Kitchener MWWTP is the largest of these plants (serving >100,000 people) and previous studies have shown that male rainbow darters (Etheostoma caeruleum) collected near this plant are feminized, having reduced fertilization success, and reduced progeny survival to hatch. While it is well recognized that MWWE has direct impacts on the sexual development and performance of the F0 generation, little is known about the consequences of MWWE exposure on subsequent generations of fish. The purpose of this study was to determine whether parental exposure history had an impact on the response of larvae to subsequent exposure. Progeny of rainbow darter collected from sites of various degrees of urban and MWWE exposure were exposed to either de-chlorinated tap water control, 10% MWWE, or 10 ng/L ethylestradiol (EE2) from 21 to 100 days post hatch (dpH). Survival, growth, and sexual maturation of larval fish were measured at 100 dpH. In general, exposure of larval rainbow darter to either EE2 or MWWE had no impact on survival. Parentage however had an impact on survival. At 21 dpH and 100 dpH, larvae whose parents were collected downstream of the Kitchener MWWTP had decreased survival compared to larvae whose parents were collected upstream of the urban area. An increase in the length, and weight of the fish was found in EE2 and MWWE exposed fish compared to the control group. In contrast, condition was significantly lower in the EE2 and MWWE exposed groups compared to controls. Additionally, larvae with parents from upstream of the urban limits had higher condition in the control treatment compared to any treatment or parental group. While exposure to EE2 resulted in a completely female population in larvae, there did not seem to be an impact of parental or MWWE exposure on sex ratio in this study. This study suggests that parental exposure has implications for progeny survival and fitness. Future work will compare the sexual development and molecular sensitivity of progeny from different parents to MWWE exposure.

MO093 Development of new microsatellites markers to investigate genetic diversity between metalloccic and non-metalloccic earthworm populations.
D. SOULEMAN, University of Lille; M. Pauwels, University of Lille / Laboratoire de Génétique et Evolutions des Populations Végétales; F. Grumiaux, LGCge / EA 4515; H. Fretot, University of Lille / Laboratoire de Génétique et Évolution des Populations Végétales; F. Vandenbulcke, LGCge, Université de Lille I / LGCge EA Univ Lille Fonctionnement des Ecosystèmes Terrestres Anthropisés
Cité Scientifique SN Rdc F Villeneuve d’Ascq France

Anthrpic activities can result in heavy metal contamination of urban and agricultural soils. Pollution may induce changes in organisms living in close contact with soil. Biological responses observed at the individual or infra-individual level of biological organization led to the development of biomarkers which consists often in evidencing biological modifications following a contaminant stress in laboratory conditions, using naïve organisms and it is sometime proposed to use the biological state of individuals from sentinel species collected in the field to evaluate the level of environmental exposure. However, considering the possibility of local adaptation following long-term exposure, response of organisms sampled in the field may substantially differ from laboratory specimens. In a recent review, we investigated this point focusing on the definition and validity of molecular biomarkers of metal pollution using earthworms of the Lumbricidae family. It appears that conditions for local adaptation to occur are present in earthworms. Some infra-individual evidence of adaptation obviously exists. Consequently, there is need for evolutionary studies investigating the level and distribution of genetic diversity in natural populations of earthworms. Therefore, it seems necessary to integrate the potential effects of local adaptation to METEs on biological responses of organisms in the development of biomarkers. Three species of Lumbricidae family belonging to two ecological groups were chosen for this study. L. terrestris, an anecic earthworm, L. rubella and L. castaneus, epigeic earthworms. In this work, we decided to investigate a possible local adaptation, owing to pollution, in earthworms from Lumbricidae family populations using microsatellite markers approach. Microsatellite markers may provide data to investigate the genetic differences between earthworm populations issued from polluted and reference sites. Eight microsatellite markers were identified in L. terrestris. Same approach of microsatellite identification is ongoing in L. rubella and L. castaneus. Microsatellite markers are essential tools to understand the evolutionary dynamics of populations and direct and indirect selective forces that shape genetics variation in natural populations. We hypothesized that probable lose of genetic variation may occur due to selection pressure and genetic drift in population below to polluted area.

Fish model species in environmental toxicity (P)

MO094 Miniaturized in-flow sorter for dispensing fertilized zebrafish embryos for ecotoxicity biotests
N.M. Fuad, J. Skommer, RMIT University / School of Applied Sciences; T. Friedrich, University of Massachusetts / Department of Molecular and Cellular Biology; J. Kaslin, Monash University / ARM; D. Wlodkowski, RMIT University / School of Applied Sciences.

The current bottleneck in using zebrafish embryos for screening purposes is a tedious manual evaluation to confirm the fertilization status and subsequent dispensing of single viable embryos to multiplate plates to perform toxicity analysis. Manual procedures associated with sorting hundreds of embryos are very monotonous and as such prone to significant analytical errors due to operator’s fatigue. This work presents the concept design of an in-flow embryo sorter capable of analysing, sorting and dispensing objects ranging in size from 1.5 to 2.5 mm such as zebrafish embryos. The major obstacle hampering sorting of millimeter sized species such as zebrafish embryos on chip-based devices is their substantial diameter (above one millimeter), mass (above one milligram), which both lead to rapid gravitational-induced sedimentation and high inertial forces. In this work, we present an innovative design of a micromechanical large particle in-flow sorter (MILIPS) capable of analysing, sorting and dispensing living zebrafish embryos for ecotoxicity testing applications. The system consisted of a microfluidic network, revolving micromechanical receptor actuated by robotic servomotor and opto-electronic sensing module. The prototypes were fabricated in polymethyl methacrylate (PMMA) transparent thermoplastic using infrared laser micromachining. Elements of MILIPS were also fabricated in an optically transparent VisiJet resin using 3D stereolithography (SLA) processes (ProJet 7000HD, 3D Systems). The device operation was based on a rapidly revolving miniaturized mechanical receptor. The latter function was to hold and position individual fish embryos for (i) interrogation, (ii) sorting decision-making and (iii) physiological sorting. The activation of the revolving receptor was performed using a Dynamixel AX12A and RX24F (Robotis Inc, Korea) robotic actuators. The system was designed to separate fertilized (LIVE) and non-fertilized (DEAD) eggs, based on optical transparency differences using infrared (IR) emitters/receivers embedded in the microfluidic chip-based system. Digital storage oscilloscope (DSO) signals were used to distinguish the differentiation characteristics of LIVE vs. DEAD embryos and a voltage threshold was set to trigger the activation of the revolving receptor.

MO095 ZebraToxChip - Microfluidic technology for automation of fish embryo toxicity (FET) biotests
F. Zhu, J. Skommer, RMIT University / School of Applied Sciences; T. Friedrich, RMIT University / School of Applied Sciences;
Fish Embryo Toxicity (FET) biotest has gained popularity as one of the most promising alternative approaches to acute fish toxicity tests. At present, the major hurdle to widespread deployment of FET in large-scale water-born toxicity projects is the lack of enabling high-throughput analytical platforms. Despite the importance and promise of FET, most experiments that test use zebrafish embryos and larvae are still performed in multi-well plates and require laborious and time-consuming manual manipulation of specimens and solutions. Furthermore, static culture of embryos and larvae can lead to a bias in toxicity studies due to e.g. drug adsorption to the surfaces and also secondary effects from the secreted embryo matrix polypeptides. Lastly, microtiter plate and oxygen delivery to embryos. This work describes design and validation of a pioneering and high-throughput Lab-on-a-Chip technology for automation of zebrafish embryo toxicity (FET) biotests performed in ecotoxicology. The innovative device supports rapid loading and immobilization of large numbers of fish embryos suspended in a continuous microfluidic perfusion as a mean of toxin delivery. The 3D high-density LOC array was fabricated in polycarbonate (PMMA) transparent thermoplastic using infrared laser micromachining while the off-chip interfaces were fabricated using additive manufacturing processes. The system’s design facilitates rapid loading and immobilization of a large number of embryos in predefined clusters of traps during continuous microperfusion of toxicants. The system supports real-time in situ analysis of developing embryos. Moreover, a miniaturized SMP USB fluorescent microscope was developed for labeling with fluorescent markers and solid-state LED illumination. A PC-computer and interconnected embedded ARM microcontroller controlled all sensor inputs and actuators outputs. This system brings us a step closer to realization of full analytical automation in ecotoxicology.

Gliotoxicity of CdCl2 in zebrafish brain

A. Monaco, A. Aurino, M.C. Grimaldi, I. Ferrandino, University of Naples Federico II / Biology

Cadmium (Cd) is known as a potent toxic metal and a significant aquatic and terrestrial environmental pollutant. In this study was verified the ability of cadmium chloride (CdCl2) to induce toxicity effects on the brain of zebrafish through the detection of the expression of glial fibrillary acidic protein (GFAP), astroglial marker. It was also carried out a staining method with methasol fast blue for the marker of astroglial cells, showed a reduction of signal in terms of intensity and area compared to that established in NOM 001 SEMARNAT (4 hours) after 2 days of treatment a decrease of GFAP expression was associated in previous works with a decrease of GAD activity in the ischemic zone of the brain of Drosophila melanogaster. Reduction in GFAP expression in the telencephalon of individuals of control showed in the telencephalon of individuals of control a faint signal. The stainings performed on cross sections of the Zebrafish telencephalon have shown tissue alterations already after 2 days of exposure to AICI3, consisting in dilations of the cerebral parenchyma and vacuoles present in pericellular area at support of possible vascular damage induced. Immunohistochemistry analysis showed in the telencephalon of individuals of control a faint signal at the level of cell bodies of nerve cells that show abundant cytoplasm. The signal was increased at the intracellular level after 2 and 7 days after start of treatment, a phenomenon that might therefore be associated with an increase in the presence of oligomers of amyloid peptide inside the cells, characteristic of the early stage of Alzheimer’s disease as well as other neurodegenerative diseases. These data confirm the toxic action exerted by AICI3 in the brain and show a correlation between this metal and existing mechanisms at the basis of neurodegenerative diseases even in this fish. The results obtained thus open the way for further analysis aimed at further confirmation of the use of this model for the study of neurodegenerative mechanisms induced by this metal.

Aluminum-Induced Alterations in Danio Rerio Development

A. Monaco, A. Aurino, M.C. Grimaldi, I. Ferrandino, University of Naples Federico II / Biology

The use of zebrafish embryos and larvae is always more widespread and accepted in the scientific community for the screening of dangerous effects exercised by processes and functionality, so it could affect the roles played by these organisms. Metals as aluminum (Al), one of the more abundant metal in the Earth's crust, have been previously associated with the development and the progression severity of neurological disorders as Alzheimer’s disease. In this study the toxicity of Al was evaluated on the development of the zebrafish larvae, paying particular attention to the nervous system. Larvae at protruding mouth stage were exposed to 24 hours continuous exposure to Al concentrations from 1 to 72 hours to test the lethality index. The mortality and the phenotypic analysis were determined after 24, 48 and 72 hours of treatment. Pericardial edema and impaired cardiac function consistent in alteration of heart rate were present in 50% of these larvae after 48 hours. A reduction of the response to stimuli in live larvae was observed from 100 μM of AICI3, just after 24 hours of the exposition and the severity of this abnormality was directly proportionate to the used concentration. In the light of these results sections of zebrafish larvae, after 48 hours of the exposure at 100 μM of AICI3, were embedded in paraffin and processed by ABC technique. The immunohistochemistry experiments performed with an antibody anti-GFAP, marker of astroglial cells, showed a reduction of signal in terms of intensity and number of positive cells, in all areas of larvae encephalon with largest decreases in the hindbrain. These data show a toxic effect exerted by Al on the development of the larvae of this teleost fish. Interesting was to observe that this metal could represent also a cardiac toxin over the neurotoxic ability that also in this study was confirmed. The decrease of GFAP expression was associated in previous works with deconstruction of cellular cytoskeleton resulting in degeneration of cellular processes and functionality of these cells and this model can represent a new way to consider for explain the neurotoxicity exercised by this metal in human brain.

Effects of exposure to metals in Zebrafish (Danio rerio) embryos

A.S. Sobrino-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia Laboratorio Alejandro Villalobos

The metals As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, V and Zn are found in high concentrations in some aquatic systems the Valley of Mexico, due to the discharges of untreated wastewater, from industrial, domestic and agricultural activities. In this paper an evaluation of the toxic effect and the degree of lipid peroxidation caused by Arsenic, Cadmium, Chromium, Copper, Mercury, Manganese, Nickel, Lead, Vanadium and Zinc metals in zebrafish embryos was performed to compare its sensitivity with Danophia magna neonates, a species that is used for the evaluation of toxicity of effluents (NMIX-AA-087-1995-SCFI), Bioassays with duration of 48 hours were performed, with ten concentrations for each metal (0.01, 0.1, 1.0, 10 and 30 ppm for Cd, Cr, Cu, Pb and Hg. And 6.25, 12.5, 25, 50 and 100 ppm for As, Mn, Ni, V and Zn) plus a control. LC50 was determined after 48 hours exposure by the Probit method. The degree of lipid peroxidation in the tissues of embryos was measured by evaluating the concentration of MDA (malondialdehyde). The metals with oxidative effects were Cd and Cu. The toxicity of these two metals increased in a non-linear fashion. The exposure to metals is considered to be a causative factor for the development of Alzheimer’s disease, a disease that affects mainly the cerebral cortex, the amygdala and the hippocampus, structures that find their counterpart in the telencephalon of Zebrafish. The mechanisms by which aluminum interacts with the nervous system, however, are understood only in part. To test the toxicity of Al on the telencephalon of Zebrafish and verify its ability to induce neurodegeneration in this fish, adult individuals were exposed for 7 days at 100 μg/L of aluminum chloride (AICI3) at pH 5.8. Brains were removed after 2 and 7 days after initiation of treatment. To evaluate the presence of histological alterations were made staining as hematoxylin-eosin and Cresyl Violet and immunohistochemical experiments performed on serial transverse sections of the forebrain by ABC method using an anti-Abeta antibody. The stainings performed on cross sections of the Zebrafish telencephalon have shown tissue alterations already after 2 days of exposure to AICI3, consisting in dilations of the cerebral parenchyma and vacuoles present in pericellular area at support of possible vascular damage induced. Immunohistochemistry analysis showed in the telencephalon of individuals of control a faint signal at the level of cell bodies of nerve cells that show abundant cytoplasm. The signal was incremented at the intracellular level after 2 and 7 days after start of treatment, a phenomenon that might therefore be associated with an increase in the presence of oligomers of amyloid peptide inside the cells, characteristic of the early stage of Alzheimer’s disease as well as other neurodegenerative diseases. These data confirm the toxic action exerted by Al on the brain and show a correlation between this metal and existing mechanisms at the basis of neurodegenerative diseases even in this fish. The results obtained thus open the way for further analysis aimed at further confirmation of the use of this model for the study of neurodegenerative mechanisms induced by this metal.
Acute, developmental and biochemical responses of Danio rerio embryos exposed to Bisphenol A: a comparative approach using Species Sensitivity Distributions

S. Silva, University of Brasilia / TOXICOLOGICAL GENETIC: R. Oliveira, University of Brasilia / Department of Genetics and Morphology; A. Andrade, Universidade de Brasilia / Gênix / Departamento de Genética e Morfologia; K. Pyk, University of Belgrade / Department of Genetics and Morphology; C.K. Grisolia, University of Brasilia / Department Genetics and Morphology. Bisphenol A (BPA) is a chemical widely used in the manufacture of polycarbonate plastics and epoxy resins. The presence of BPA in aquatic ecosystems is often associated to discharge on municipal wastewater effluents and leachate from landfills. Considering previous measurements of environmental concentrations of BPA in the Parana River (Brazilian-Paraguay), the present study intends to contribute to fill the gap on BPA toxicity to fish species considering a tropical scenario. Here, we studied the toxic effects of BPA on Danio rerio embryos using different endpoints (mortality, enzymatic biomarkers, development, and vitellogenin- VGT). The results were compared among several fish species by means of a Species Sensitivity Distributions. The fish embryos assays were based on the OECD protocol No. 236. For each assay, test solutions were prepared by successive dilution of the stock (5 mg/L - BPA 99% of purity from Sigma Aldrich). In order to evaluate the BPA stability, exposure solutions (400 ml in quadruplicate) were spiked with BPA and placed at the same experimental conditions of the ecotoxicological tests. BPA quantification was performed daily using the high performance liquid chromatography coupled to fluorescence detector. Embryo toxicity test was evaluated with newly fertilized eggs exposed to 1, 2, 4, 10, 21, 46 and 100 mg/L of BPA. A similar test was performed for collection of embryos for biomarker analyses, using sub lethal BPA concentrations (0.35, 2.02, 11.68, 67.45, 389.63 and 2250.6 µg/L). The tests were carried out at 26 ± 1 °C, 12 h light for 96 hours. Over 50% of nominal concentrations of BPA were detected in the test solutions during 4 days, indicating low degradation of the compound. After 48h exposure to the highest concentration (11.68 mg/L), pigmentation and craniofacial formation (LOEC = 11.68 µg/L) were also observed. Alterations in the enzymatic level were noticed for embryos exposed to low BPA concentrations. VTG alterations observed were compared among several fish species and seem to be the most sensitive endpoint to BPA (HC5 < 10 µg/L) in acute scenario (1-10 days) evaluation. In this study, we observed that BPA showed low acute toxicity to Danio rerio embryos, however, sub lethal ecotoxicological research on BPA indicates the necessity to regulate its use and control the concentrations of this contaminant in aquatic environments.

MO101

Biological activity and transcriptomic alterations induced by particle-bound Polycyclic Aromatic Hydrocarbons from the Mediterranean and Black Seas atmosphere.

S.R. Mesquita, Center of Marine and Environmental Research CIRM / METOX Coastal and Marine Environmental Toxicology; J. Dachs, IDAEA-CSIC / Environmental Chemistry; B.L. van Drooge, C. Barata, CSIC / Environmental Chemistry. Particle-bound Polycyclic Aromatic Hydrocarbons (PAHs) represent one of the most toxic contaminants in the aquatic environment due to their potential toxic burden of ambient PM to the aquatic biota is still unstudied. On the present study, we evaluated the particulate PAHs concentrations. No phenotypical effects on the ZET assay - RYA activity, were maximal for the effects accurately when using mixtures of drinking water containing PAHs. The results of this study may be used to fill the gap on the impact of PAHs on aquatic organisms. The European commission stressed the need for the evaluation of mixtures in the ecotoxicology. The aim of this study was to evaluate the response of zebrafish embryos to mixtures of PAHs in order to understand the effects of mixture design and to assess the impacts of mixture design on the toxicity of PAHs.

MO102

Histopathological changes of the gills of common carp (Cyprinus carpio L.) as a marker of environmental conditions in pond aquaculture

B. Raskovic, University of Belgrade / Institute of Animal Science; M. Stankovic, Z. Dulic, University of Belgrade / Faculty of Agriculture; I. Zivic, University of Belgrade / Faculty of Biology; Z. Markovic, V. Polekic, University of Belgrade / Faculty of Agriculture

For decades, histopathological parameters (HP) were used as a pollution marker in ecotoxicology. The method is cheap, reliable and provides good insight into morphology of vital organs. The results obtained usually correlate with the pollution state of investigated ecosystem. We used HP changes of the gills of common carp (Cyprinus carpio L.) in order to monitor general fish health in pond aquaculture using a semi-quantitative system. In this system, fish is reared in earthen pond; water supply is from a common lake and pond water is managed and man-maintained, the water quality is monitored. A field studies were conducted to compare gill HP of carp reared on 3 fish farms with different water supply: one was considered as a control - a fish farm of the Faculty of Agriculture in Belgrade school estate (CON), supplied with well water; other two commercial farms used water from nearby currents. Water supply for Kanjiža fish farm (KAN) was from the Tisza River, while for the fish farm Vrsacki Ritovi (VRTX) it was Danube-Tisza-Danube canal. During one growing season, one-year common carp juveniles were collected every fortnight, from the same ponds of each farm. The second gill arch was fixed in 4% formaldehyde, and processed in routine HP protocol, stained with hematoxylin and eosin. The frequencies of lesions were calculated and compared between farms using Mann-Whitney U test. Water samples were also taken fortnightly: temperature, pH, DO, ammonium ions, and nitrates were measured. The fish at all fish farms showed a distinctive HP response to pond conditions: focal hyperplasia, lifting of the respiratory epithelium and hyperemia of gills prevailed. The majority of other lesions were circulatory changes: aneurism, blood stasis and lamellar telangiectasia. Severe changes, such as necrosis or erosion (EA) and independent lesions (LA), Berenbaum (1985). Analysis of HP results between farms provided clear difference: all changes were lower at CON, compared to KAN and VRT. VRT fish had higher frequencies of majority of lesions compared to KAN. Water quality did not differ greatly between farms. Differences in studied ponds ecosystems and rearing conditions (stocking density and dimension of the ponds) including possible sub lethal pollution coming from the farm water supply could explain differences in gill HP and indicate risks for the reared fish health status, and therefore production result.

MO103

Mixture toxicity analysis of water contaminants with the fish embryo assay

S. Schneider, Helmholtz Centre for Environmental Research - UFZ; W. Busch, Helmholtz centre for environmental research, UFZ; Bioanalytical Ecotoxicology; R. Altenburger, UMF Centre for Environmental Research / Department of Bioanalytical Ecotoxicology; E.K. Kuester, Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology

The European commission stressed the need for the evaluation of mixtures in the ecotoxicology. Numerous studies, in many cases the contaminants are regulated on a single substance basis and not as mixtures (with certain exceptions such as pesticides and PAHs, EU Water Framework Directive Nr. 2000/60/EC). As multiple contaminants with similar mode of action are in solution in the water at the same time and can have combined effects these would be underestimated by single substance evaluation only. Beside the existence of the two main concepts of action: concentration addition (CA) and independent action (IA), Berenbaum (1985) suggested to use CA as the universal assessment tool for joint action. This was supported amongst others by Backhaus and Faust (2012). Due to the high number of known water contaminants many substances have to be evaluated in a short amount of time. The risk evaluation can be focused on safe guarding of human health (Grummt et al., 2013, WHO 2010) or the environment. One major organism group which is often the focus of evaluation is fish. Therefore it is important to test fish embryos exposed to BPA. A test on fish embryos exposed to 0, 1, 2, 4, 10, 21, 46 and 100 mg/L of BPA toxicity test was evaluated with newly fertilized eggs exposed to 1, 2, 4, 10, 21, 46 and 100 mg/L of BPA. A similar test was performed for collection of embryos for biomarker analyses, using sub lethal BPA concentrations (0.35, 2.02, 11.68, 67.45, 389.63 and 2250.6 µg/L). The test was carried out at 26 ± 1 °C, 12 h light for 96 hours. Over 50% of nominal concentrations of BPA were detected in the test solutions during 4 days, indicating low degradation of the compound. After 48h exposure to the highest concentration (11.68 mg/L), pigmentation and craniofacial formation (LOEC = 11.68 µg/L) were also observed. Alterations in the enzymatic level were noticed for embryos exposed to low BPA concentrations. VTG alterations observed were compared among several fish species and seem to be the most sensitive endpoint to BPA (HC5 < 10 µg/L) in acute scenario (1-10 days) evaluation. In this study, we observed that BPA showed low acute toxicity to Danio rerio embryos, however, sub lethal ecotoxicological research on BPA indicates the necessity to regulate its use and control the concentrations of this contaminant in aquatic environments.

MO104

Modelling target gene expression profiles in zebrafish Danio rerio exposed to Microcystis aeruginosa and the toxin microcystin-LR

D.A. Sabrei, Faculty of Life Sciences; C.H. Redshaw, European Centre for Environment and Human Health and University of Plymouth / School of Geography Earth and Environmental Sciences; A.J. Moody, University of Plymouth; T.B. Henry, Heriot-Watt University / School of Life Sciences

Microcystis aeruginosa is a cyanobacterium that can produce the hepatotoxin

microcrystin-LR (MC-LR) and that is responsible for many harmful algal blooms (HABs) in freshwater. Blooms of *M. aeruginosa* frequently occur in eutrophic surface waters, and the presence of the cyanobacteria and toxins can become an important problem for ecological and human health. We have found that toxicity in the zebrafish *Danio rerio* includes effects that are independent of the toxin. Our objectives are to investigate changes in target gene expression profiles in zebrafish in response to copper nitrate solutions in conjunction and duration of exposure to *M. aeruginosa* and MC-LR. We have evaluated the toxicity and effects of both strains at both concentrations of *M. aeruginosa* to generate a supply of lyophilized cyanobacterial cells characterized for use in toxicity tests. Zebrafish larvae (age 72 h post fertilization) will be exposed to lyophilized *M. aeruginosa* or MC-LR at MC-LR concentrations of (5, 25, 50 μg/L) and fish will be sampled after different exposure durations (2, 4, 8, 24, 48 h). Later is essential to fish but can be toxic in high concentrations thus, due to its abundance on earth, it is a metal that can contaminate the environment. Changes in the diversity of fish in the world, and their distribution across the world, are used as indicators of environmental health. Early life stages are very sensitive to environmental factors as potential additional stressors to aquatic life. Changes in the expression of genes involved in cellular protection against toxicants, such as copper ions to zebrafish early life stages and quantify biochemical biomarkers (GST, LDH), can be used to assess the impact of metal ions contamination on aquatic organisms. Metal ions contamination are a major source of concern around the world. To understand the molecular responses of fish during the progression of an exposure to a HAB caused by *M. aeruginosa*.

**MO105** Effects of uptake of Copper Nitrate Trihydrate on zebrafish (*Danio rerio*) early-life stages

K.R. Silva, A.J. Nunes, University of Aveiro / Department of Biology; C.E.S.A.M.; A. V.X. National de Pesquisas da Amazonia INPA

Climate change studies have highlighted the importance of including environmental factors as potential additional stressors to aquatic life. Changes in climate and hydrological extremes have been observed in the last fifty years while projections of climate models raise special concern for large areas of tropical regions principally in developing countries. The Amazon Basin holds the greatest diversity of fish in the world, during their development and a non-tropical region of habitats in the Amazon region is defined by the complex organism-environment interactions. In the Amazon region there are major sources of pollution due to anthropogenic activities, namely associated with high concentrations of metals reaching water bodies. Metal ions contamination are a major source of concern around the world. Copper is essential to fish but can be toxic in high concentrations thus, due to its abundance on earth, it is a metal that can contaminate the environment. It is known to cause toxic effects in aquatic organisms although risks to tropical fish are not well known. The objective of this study is to evaluate the acute toxicity of Copper ions to zebrafish early life stages and quantify biochemical biomarkers (GST, LDH and CHE) as indicators of exposure to these Metal. The exposures were conducted following the OECD’s protocol for the Fish Embryo Toxicity (FET) Test (OECD, 2006). To determine the toxicity of metal for early-life stages of zebrafish the following treatments were used: 0 (control), 0.038, 0.06, 0.095, 0.15, 0.24 and 0.38 mg L⁻¹ of copper in water (prepared as copper nitrate solutions). Biomarkers’ activities (GST, ACHE and LDH) were measured in early-life stages after exposure to concentrations of Copper corresponding to the LC1, LC2, LC5 and LC10. The lethal concentration for 50% of the population after 96 hours of exposure (LC50₉₆h) was calculated with the help of ToxCalcMix spreadsheet running under Microsoft Excel software. One-Way ANOVA tool was used for data analysis of enzyme activity with SigmaPlot 11.0. LC50₉₆h for the zebrafish early-life stages. It can be concluded that acute toxicity data obtained from zebrafish indicated sensitivity to toxicants like blood circulation, spontaneous movement, heartbeat and early hatching. Based on our results, the second-generation antihistamines have less toxicological effect than the first generation antihistamines. For example, we observed a factor of 66,7 higher. A noticeable difference was observed in blood circulation. There was a significant effect after exposure to first-generation antihistamines Diphenhydramine after 48 hpt at 120 μM. The second-generation antihistamine Cetirizine had no effect even at 2 mM. Calculated with a safety factor of 400 the first generation antihistamines Diphenhydramine had a LOEC of 15 μM in comparison to Cetirizine with a LOEC of 1000 μM. Toxicity is a factor of 66,7 higher. A noticeable difference was observed in blood circulation.

**MO106** Morpho-functional and transcriptional response of zebrafish retinal cells to cadmium stress

R. Sandoval, Department of Biology; R. Panzuto, Biologia; M.G. Espostio, University Federico II of Naples; P. Simonelli, GSI Helmholz Center for Heavy Ion Research / Department of Biophysics; B. Avallone, University Federico II of Naples; C.M. Motta, University Federico II of Naples / Department of Biology

Ocular malformations are observed in embryos and adults of aquatic species after exposure to toxicants. In this study, cadmium, a chemical stressor, was used to determine the OECD’s protocol for the Fish Embryo Toxicity (FET) Test (OECD, 2004). To determine the toxicity of metal for early-life stages of zebrafish the following treatments were used: 0 (control), 0.038, 0.06, 0.095, 0.15, 0.24 and 0.38 mg L⁻¹ of cadmium in water (prepared as cadmium chloride solutions). The developed tool was used to assess the impact of metal ions contamination on aquatic organisms. Metal ions contamination are a major source of concern around the world. To understand the molecular responses of fish during the progression of an exposure to a HAB caused by *M. aeruginosa*.

**MO107** Sublethal toxic effects of first- and second-generation antihistamine in *Danio rerio* for a tentative environmental risk assessment

K. Kroll, Unv. of appl. sciences Northwestern Switzerland; A.K. Zenker, University of Appl. Sc. Northwestern Switzerland / Diphenhydramine

Histamine antagonists were developed over 80 years ago. They inhibit the action of histamine by blocking to the receptor. There could be also an inhibition on the transformation of histidine to histamine. Histamine is known for its role in allergic reactions and their symptoms. In total there are up to 30% of the Swiss and 2,3 % of the European population having trouble with hay fever or other allergies. In total there are 2.4 million people in Switzerland over 12 years old suffering from allergy and many people affected by allergies are expected in the future. All of them are forced to use antihistamine, whereby high consumption rates of theses pharmaceuticals could pose a threat to the environment. For example after ingestion 70% of Cetirizine is not metabolised in the human body, is then excreted and will enter surface water via treated wastewater. Today there are only a few tests available to study the risk of a pharmaceutical in the environment. This study was wanted to know whether only the pharmacological effects of second generation antihistamine were improved or also their impact to the environmental compared to first generation drugs. Since first-generation antihistamines are responsible for many side-effects like increasing heart rate or fatigue, we tested in this study the sublethal generation antihistamine diphenhydramine and the often used second-generation antihistamine cetirizine. *Danio rerio* embryo toxicity test was used and expanded to 144 hpf. Organs under investigation were liver, heart, otic vesicle, swim bladder, eyes, lateral fin, trunk, belly and other visible changes like blood circulation, spontaneous movement, heartbeat and early hatching. Based on our results, the second-generation antihistamines have less toxicological effect than the first generation antihistamines. For example, we observed a factor of 66,7 higher. A noticeable difference was observed in blood circulation. There was a significant effect after exposure to first-generation antihistamines Diphenhydramine after 48 hpt at 120 μM. The second-generation antihistamine Cetirizine had no effect even at 2 mM. Calculated with a safety factor of 400 the first generation antihistamines Diphenhydramine had a LOEC of 15 μM in comparison to Cetirizine with a LOEC of 1000 μM. Toxicity is a factor of 66,7 higher. A noticeable difference was observed in blood circulation.

**MO108** Sub-lethal effects of Carbaryl and Carbendazim to zebrafish embryos

K. Kroll, University of Aveiro, Department of CESAM / Biology; J. Henriqueis, Universidade de Aveiro / Department of Biology; A.R. Almeida, University of Aveiro / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; I. Domingues, University of Aveiro / CESAM Department of Biology

There is strong evidence that the use of pesticides has major impacts in non-target wildlife with serious consequences to aquatic ecosystems. Typically environmental concentrations of pesticides rarely cause lethality; therefore the risk assessment of these chemicals is better achieved if a battery of sublethal endpoints is used. Within this context, the main goals of this work are to evaluate the effects of Carbaryl – a carbamate insecticide – and Carbendazim – a benzimidazole carbamate fungicide – on the first generation antihistamine cetirizine. For the calculation of the effective concentration that caused toxicity, we used a factor of 2.22 and 0.13 for Cetirizine. For the calculated environmental risk, Diphenhydramine was 9,48 times higher compared to Cetirizine.
of considering the sublethal effects of environmental contaminants in risk assessment in order to better estimate long term effects of contaminants in aquatic ecosystems.

**MO110**

Zebrafish embryo test used in the effects assessment of three classes of antidepressants consumed in Brazil - serotonin selectives reuptake inhibitors, monoamine oxidase inhibitors, and tricyclic

D.S. Moura, Universidade de Brasília / Genética e Morfologia: J.T. Souza, University of Brasília / Departamento de Genética e Morfologia Instituto de Biologia; N.O. Farias, R.C. Oliveira, Universidade de Brasília / gtx / Departamento de Genética e Morfologia Instituto de Biologia; C. Lisboa, University of Brasilia / Departamento de Genética e Morfologia; D. Gagnon, Universidade de Brasília / gtx / Departamento de Genética e Morfologia Instituto de Biologia; L.B. Ferreira, Universidade de Brasília / gtx / Departamento de Genética e Morfologia Instituto de Biologia; R. Oliveira, Universidade de Brasilia / Departamento de Genomics and Morphology; C.K. Grisolia, University of Brasilia / Department Genetics and Morphology

Antidepressants (ATD) are a large prescriced groups of pharmaceuticals, mainly used on treatment of depressive syndromes and anxiety. ATD acts on the central nervous system temporarily changing brain function, behaviour and consciousness. ATD have three principal classes: tricyclic, serotonin selective reuptake inhibitors (SSRI) and monoamine oxidase inhibitors (MAOIs). After administration, ATD can be metabolized, biotransformed, conjugate and excreted in urine and faeces. The excreted amount of antidepressants/metabolites will depend on the type of compound, dosage, route of application. Once excreted, the ATDs will reach aquatic ecosystems mainly through domestic effluents. It makes the domestic sewage the major source of ATD for water contamination. This occurs due to the low removal rate of several ATD in sewage water treatment plants. In this scenario, the present study aims to evaluate the acute and embryotoxicity of different ATD, namely fluoxetine (FLX, Generic), sertraline (SERT, Generic), agomelatine (AGOM, Generic), mirtazapine (MIR, Generic) and bupropion (BPP, Generic). To do so, zebrafish embryos were used as model organisms. Toxicity tests were performed based on the Fish Embryo Toxicity test OCDE n° 236. The organisms were exposed to 2 mL of solution of at least six concentrations, including a negative control group. All tests were performed in a climatic chamber (26 °C and 12 h of light) with a minimum of 4 days. The concentrations ranged from 0.1 - 15 mg/L for FLX, 0.007 - 0.24 mg/L for SERT, 1 - 100 mg/L for AGTL, 4,68 - 300 mg/L for BPP, 1 - 100 mg/L for MIR, 1 - 73 mg/L for NTB. Based on the obtained results, and considering only the mortality, a following toxicity ranking could be stablished: SERT (96h-LC50 = 0,022 ± 0,001 mg/L), FLX (96h-LC50 = 6,05 ± 0.19 mg/L), BPP (96h-LC50 = 11.62 ± 0.98 mg/L), AGOM (96h-LC50 = 4,75 ± 0.00 mg/L), MIR (96h-LC50 = 29.61 ± 0.00 mg/L), and NTB (96h-LC50 = 30.72 ± 0.01 mg/L). ATD exposure caused changes in the development of zebrafish (tail deformities, lack of equilibrium, and hatching delay). Lethal and embryotoxic effects observed in this study can occur in low doses (e.g. SERT case) indicating a high risk of exposure to antidepressants. Future studies should focus on chronic toxicity of ATD for fish and the toxicity to aquatic organisms belonging to other trophic levels.

**MO111**

Total and differential count of blood cells of Oreochromis niloticus exposed to azithromycin in chronic toxicity and efficacy tests

N.S. Shiogiri, DCF; C.V. Befuti, FCAV UNESP; S. Carraschi, C. da Cruz, São Paulo State University / School of Medicine; C.P. Grisolia, Universidad Federal de Sao Carlos / Ciencias Fisiologicas

The intensive culture of fish is a sector in expansion and is highly vulnerable to infectious diseases. The intensive use of antibiotics to control bacterial diseases may cause negative impacts to the aquatic environment, including to fish. So, the aim of this study was to evaluate chronic effects on the total erythrocytes and the percentage of leukocytes in tilapia (Oreochromis niloticus) (Wt. 70 - 100 g) exposed to 1, 50 and 100 mg L⁻¹ of azithromycin (AZT). The AZT were previously diluted in ethanol then, two control groups (with and without ethanol) were used. The exposure system was static, three replicates and lasted 14 days. In addition, was performed an experiment of efficacy of AZT, where fish were treated with AZT administrated by food for five days. Fish was infected with the bacteria Aeromonas sp. (6.2 x 10⁶ UFC mL⁻¹) and the treatments were health control, health control that received food with AZT, control with saliné 7%, infected untreated fish, and AZT treatments with 100 mg and 125 mg of AZT per kilo of food. Total erythrocytes and the percentage of leukocytes were analyzed. In the chronic toxicity experiment, the number of thrombocytes and leukocytes presented a significant increase in the treatment of 100 mg L⁻¹ compared with the control group with and without ethanol. The differential count presented an elevated percentage of lymphocytes compared to other leukocytes types (monocytes, basophils, eosinophil, neutrophils and immature leukocytes) in all concentrations. The leukocyte cells presented similar data in all concentrations. In the efficacy experiment, the number of thrombocytes increased significantly in the treatment of 125 mg AZT Kg⁻¹ when compared to the health control. The total leukocyte number did not present significant difference. The differential count of leukocytes presented elevated percentage of lymphocytes, but in infected untreated, the percentage of lymphocytes decreased and the percentage of neutrophils increased, proving that an infection was happening, since this type of cell is the first to appear in an infection site. In chronic exposure, the levels of thrombocytes and leukocytes increased indicating that AZT, at the highest concentration studied, can stimulate this system, but among the different kind of leukocytes didn’t have difference indicating that there was no infection happening. In the efficacy test was observed that the AZT did not affected this immune system. Financial support: FAPESP Proc. 2011/21552-6.

**MO112**

Relationships in trace metals between waters, tissues and otoiloh of juvenile flounder: a 3-month experimental approach to validate the use of otoiloh as natural tracer of metal contamination

j. selleslagh, a. échard, Université Bordeaux; P. Gourves, Université de Bordeaux 1; M. Baudrion, Université Bordeaux 1; f. daferat, Irstea Bordeaux

The flounder is one of the most commercially important flatfish of the Mediterranean Sea. The intensive use of antibiotics to control bacterial diseases may cause negative impacts to the aquatic environment, including to fish. So, the aim of this study was to demonstrate the potential use of otoiloh as natural tracer of metal contamination. Thus, bioaccumulation of metal (cocktail of Cd, Pb and Ni) in tissues (liver, kidney and gills) and otoiloh of 230 juvenile flounders (Platichthys flesus) were analysed after increasing levels (natural, 5-time, 10-time and null) along a 3-month direct exposure under controlled conditions. Chemical analyses were carried out by ICP-OES and GTA-AAS for water and tissues and otoiloh chronologies were performed by atomic absorption. The number of treatments with 100 mg and 125 mg of AZT per kilo of food. Total erythrocytes and thrombocytes didn’t have difference indicating that there was no infection happening.
physiological and biomedical research and its genome was recently sequenced. With a full generation time of ≤37 days, *N. furzeri* offers a great potential to perform time- and cost-efficient long-term and multigenerational ecotoxicological studies compared to other fish-model species (zebrafish 60-75 days, medaka 70-81 days). Another advantage is their production of drought resistant dormant eggs that can be stored ‘on the shelf’, overcoming the need and costs of a continuous culture. Whenever needed, fish can be retrieved without damaging the eggs. The prospect of **N. furzeri** has not sufficiently explored yet in ecotoxicological studies partly due to the continuing lack of important basic ecotoxicological information. Therefore, we here aim at providing an initial assessment of this promising ecotoxicological model. The sensitivity of **N. furzeri** to copper (Cu(NO$_3$)$_2$, 3H$_2$O) was assessed in an acute and chronic toxicity test. In these experiments, fish were continuously exposed to concentrations of copper starting from 48h after hatching. In the acute toxicity test, mortality and buoyancy were scored to quantify LC$_{50}$ and EC$_{50}$ values during an exposure period of 14 days. The chronic toxicity exposure lasted for the whole lifetime and effects on growth and reproduction were quantified. Results of the acute toxicity test with **N. furzeri** demonstrate an overlap in sensitivity range with that of current fish models in ecotoxicology. In addition, long-term exposure to a sublethal concentration (inferred from the acute test) resulted in 100% mortality after 3 weeks. These concentrations also had a significant effect on growth and maturation time. Overall, through its unique trait-set (short maturation time and dormant eggs) and a sensitivity to a well studied toxicant that is comparable to conventional fish models, **N. furzeri** could rapidly be developed as a valuable ecotoxicological model for chronic and multigenerational toxicological risk assessment. To draw general conclusions in terms of fish in the experiment with different types of pollutants will however be needed.

**MO115**

**Olfactory toxicity in fish - a scarcely regarded topic**

S. Vez, RWTH Aachen University; H. Hollert, RWTH Aachen University / Institute of Aquatic Toxicology

According to the German Federal Environmental Agency, in Germany alone about 43,000 tons of pesticides are sold and 2.5 kg of active ingredient are applied per hectare agricultural surface each year. By avulsions following heavy rain or via drainage systems these pesticides can find their way into the aquatic environment and as they often exhibit a lack of target specificity, they could negatively affect the aquatic flora and fauna. For fish, the olfactory system is of great importance, as it mediates a variety of essential activities and behaviors, for instance food detection, kin recognition, homing, the predator response and mating habits. Consequently, an impairment of olfaction can affect individual fish as well as whole populations. The olfactory epithelium of fish is located in two olfactory pits, which are flooded constantly by the surrounding water. The olfactory receptor neurons, which are responsible for the detection of odors, are situated within the olfactory epithelium. These bipolar neurons are only separated from the surrounding water by a thin layer of mucous. In such an exposed situation, dissolved xenobiotics can interact with them as easily as natural odors do. In the last years some studies concerning the impact of xenobiotics on fish olfactory have shown that several metals and pesticides are able to influence the olfactory system of fish in environmentally relevant concentrations. Despite the crucial role the olfactory system plays in fish, to date there are only few studies addressing an impairment of the latter. Neither does the European regulation of chemicals include risk assessment concerning olfactory toxicity in fish. Furthermore very little is known about the mechanisms underlying an olfactory impairment. Hence, there is a great need for understanding about the mechanisms underlying an olfactory impairment. Hence, there is a great need for understanding about the mechanisms underlying an olfactory impairment. However, in general, there is a need for understanding about the mechanisms underlying an olfactory impairment. However, in general, there is a need for understanding about the mechanisms underlying an olfactory impairment. Furthermore very little is known about the effects of ocean acidification. The information obtained on this mechanism could be identified by trace elemental profiles and concentrations. Elemental quantification was carried out by combining PIXE (particle induced X-ray emission) and RBS (Rutherford backscattering spectrometry). Major brain regions were identified using both mass density variations and elemental distributions, having optical micrographs of stained sections as reference. Cellular arrangements could be identified by trace elemental profiles and concentrations. Elemental maps, namely Cu, Fe and Zn, to elucidate the grey matter, white matter and cellular arrangements in eel exposed to different environmental conditions that highlight homeostasis modification, altered permeability of the blood-brain barrier and suggest risk for neurological toxicity. The inventory of physiological measures containing images and elemental compositions of brain regions of fish exposed to different environmental conditions will help defining total and local brain vulnerability to metals and pollution load levels.

**MO117**

**Integration of multi-level biomarker responses of the pale chub exposed to wastewater effluents in Miho stream**

W. Kim, Korea Institute of Toxicology (KIT) / Environmental Toxicology Research Group, S. Lee, Korea University / downstream site; J. Jung, Korea University / Environmental Science and Ecological Engineering

This study evaluated the effects of an wastewater treatment plant (WWTP) effluents on multi-level biomarker responses at molecular, biochemical, histological and physiological levels in freshwater pale chub, Zacco platypus. At the same time, the contents of polycyclic aromatic hydrocarbons (PAHs), perfluorinated organic compounds (PFCs), alky phenols and heavy metals in the surface water and in the liver of fish at biomonitoring sites were measured. Heavy metal Cu ranged 1.4-1.8 µg/L, Zn ranged 1.7-5.6 µg/L, but other metals, PAHs, PFCs, alkyl phenols did not detected. Our findings showed that various molecular and biochemical or physiological response were significantly increased at the downstream site compared to those of the upstream, and these responses primarily depended on accumulated metal concentrations. In addition, significant changes to the liver somatic index at the physiological level were observed in downstream. The current findings demonstrate that the primary causes affecting fish health in the downstream site are through cascade of adverse effects caused by accumulated heavy metals from effluents. According to multivariate analysis, oxidative stress markers and physiological parameters were key biomarkers to contribute in effluent site discrimination. Wild larvae of this species was tested in the bioaccumulation of combined biomarkers and multi-level biomarker responses could offer great promises for providing information that can contribute to effluent assessment.

**MO118**

**Evaluating the effects of ocean acidification on sand-smelt larvae: a case study addressing trophic biomarkers and swimming ability**

C.S. Esteves da Silva, Polytechnic Institute of Leiria / Marine Resources Research Group; S.C. Novaes, Polytechnic Institute of Leiria / ESTM and GIRM; A.R. Lopes, Faculdade de Engenharia - Universidade do Porto / LEPABE Laboratório de Engenharia de Processos Ambiente Biotecnologia e Energia; M.F. Lemos, Instituto Politécnico de Leiria / Dept Biology; E.J. Gonçalves, A.M. Faria, ISPA/Instituto Universitário de Engenharia de Processos Ambiente Biologia Logística / Politécnico de Leiria

Although ocean acidification is a critical global problem known for decades, scientific investigation of its effects is still in its infancy. To date, most research has been conducted on calcifying organisms and little attention has been given to the potential impact on other ecosystem processes and components like early life-stages (larvae and juveniles) of non-calcifying organisms. Due to their vulnerable physiological state, small body size and high sensitivity to environmental variation, the effects of acidification are most likely to be detected at these early developmental stages. The main aim of this study was to investigate the effects of three different concentrations of pCO$_2$ in the early stages of sand-smelt, Atherina presbyter, a temperate fish species common in Atlantic European coastal waters. Wild larvae were collected in mid-July 2018 in and caught in Arrábida Marine Park, Portugal and were kept in controlled conditions with different pCO$_2$ levels (Control: ~ 400 µatm; Medium: ~ 1000 µatm; High: ~ 1800 µatm) between 7-10 days. The swimming ability ($U_{sw}$) of larvae was evaluated as a behavioural endpoint, together with biochemical biomarkers related with oxidative stress (superoxide dismutase, catalase, lipid peroxidation, DNA damages) and other physiological markers (RNA expression of EROD, deethylase (EROD) enzymatic activity at the biochemical level biomarker responses at molecular, biochemical, histological and physiological levels in freshwater pale chub, Zacco platypus. At the same time, the contents of polycyclic aromatic hydrocarbons (PAHs), perfluorinated organic compounds (PFCs), alky phenols and heavy metals in the surface water and in the liver of fish at biomonitoring sites were measured. Heavy metal Cu ranged 1.4-1.8 µg/L, Zn ranged 1.7-5.6 µg/L, but other metals, PAHs, PFCs, alkyl phenols did not detected. Our findings showed that various molecular and biochemical or physiological response were significantly increased at the downstream site compared to those of the upstream, and these responses primarily depended on accumulated metal concentrations. In addition, significant changes to the liver somatic index at the physiological level were observed in downstream. The current findings demonstrate that the primary causes affecting fish health in the downstream site are through cascade of adverse effects caused by accumulated heavy metals from effluents. According to multivariate analysis, oxidative stress markers and physiological parameters were key biomarkers to contribute in effluent site discrimination. Wild larvae of this species was tested in the bioaccumulation of combined biomarkers and multi-level biomarker responses could offer great promises for providing information that can contribute to effluent assessment.

**MO119**

**Endocrine disrupting effects of TritanTM copolyester using Oryzias latipes, a temperate fish species common in Atlantic European coastal waters**

B. Tindall, University of Exeter / Department of Biology; S. Shaheen, University of Exeter / Department of Biology; A. Clark, University of Exeter / Department of Biology; J. Zalkowsky, University of Exeter / Department of Biology; C. Hafeez, University of Exeter / Department of Biology; N. Harman, University of Exeter / Department of Biology; P. Leek, University of Exeter / Department of Biology; S. Brenchley, University of Exeter / Department of Biology; J. Garside, University of Exeter / Department of Biology; D. Thomas, University of Exeter / Department of Biology; J. Zhang, University of Exeter / Department of Biology; M. Wang, University of Exeter / Department of Biology; T. Zhao, University of Exeter / Department of Biology; W. Kim, Korea Institute of Toxicology (KIT) / Environmental Toxicology Research Group, S. Lee, Korea University / downstream site; J. Jung, Korea University / Environmental Science and Ecological Engineering

This study aimed to evaluate the potential for providing information that can contribute to effluent assessment.
A. Felicio

Effects of Diuron and its main biodegradation products to Nile tilapias, in the Grant Agency of Czech Republic P503/11/11 "CENAKVA" (No. CZ.1.05/2.1.00/01.0024) and "CENAKVA II" (No. LO1205). The study was financially supported by the MEYS of the CR.

Rainbow trout. At environmentally relevant level, DIL affected the biochemical endpoints at environmentally relevant concentration in the present study. However, growth, survival, and changes in gene expression were observed after the 40 d exposure to CHDM and TPA. Human ardenocarcinoma (H295R) cell line and GH3 cell line were also employed for the quantitative evaluation of xenobiotic effects on steroidogenesis and thyroid endocrine system, respectively. In early-life stage exposure to O. latipes, significant decrease of juvenile survival and weight were observed in fish exposed to 10 μg/L and ≤1 mg/L CHDM, respectively. Expressions of cyp9b, cyp19a, and era mRNA in fish exposed to CHDM were significantly up-regulated in a dose-response manner. The results of H295R cell assay also showed that both chemicals could alter steroidogenic pathway and increase estrogenicity. CHDM and TPA did not cause significant effects on proliferation rate of GH3 cells in the range of concentration tested. Given the extensive use of tritam™ and their loadings to the environment, their long-term environmental regulators and the environmentally relevant concentrations deserve further studies. Keywords: Early life stage test, H295R cell, GH3 cell, Tritan Acknowledgement – This study was supported by National Research Foundation of Korea (NRF, Project no. 2013R1A1A01016684).

MOI20

Effects of selected PhAs at environmentally relevant concentrations on rainbow trout (Oncorhynchus mykiss) T. Randak, University of South Bohemia in Ceske Budejovice Faculty of Fisheries and Protection of Waters / Laboratory of Environmental Chemistry and Biochemistry; V. Burkina, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydrogenoses Vodnany Czech Republic; C. Steinbach, University of South Bohemia in Ceske Budejovice; K. Grabicova, University of South Bohemia in CB; R. Grbic, University of South Bohemia in CB / Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrogenoses; G. Fedorova, University of South Bohemia in CB / Faculty of Fisheries and Protection of Waters at South Bohemian Research Center of Aquaculture and Biodiversity of Hydrogenoses Vodnany Czech Republic; V. Zlabek, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters LECHB

In recent years, potential risks associated with the release of pharmacologically active compounds (PhAs) into the aquatic environment have become an increasingly important issue due to the environmental regulations and the environment. The aim of this study was to find the effects of selected PhAs mainly at environmentally relevant concentrations on rainbow trout (Oncorhynchus mykiss). Four representatives of the most used classes of human pharmaceuticals atenolol (AT), verapamil (VRP), clotrimazole (CLO) and dilatazem (DIL) were chosen for this study. Selection was based on the consumption, published data and our screening studies performed in surface water. Chronic toxicity tests with individual pharmaceuticals were performed. The tested concentrations included environmentally relevant concentration, 1% 96 LC 50 and 10% 96 LC 50. The test fish were sampled after 21 and 42 days of exposure. In these tests, the effects of fish were used following endpoints: haematological parameters and biochemical profile of blood, oxidative stress biomarkers and antioxidant enzymes activities, histology, vitellogenin concentration in blood plasma, enzymatic activity of CYPs in liver and chemical analyses. At the environmentally relevant concentration, AT caused a decrease in the haemoglobin concentration, an increase in the lactate content of the blood plasma and congestion suggesting a reduction in oxygen supply. Although we did not observe any effects of VRP on studied endpoints, significant decrease of juvenile survival and weight were observed in fish exposed to 10 μg/L and ≤1 mg/L CHDM, respectively. Expressions of cyp9b, cyp19a, and era mRNA in fish exposed to CHDM were significantly up-regulated in a dose-response manner. The results of H295R cell assay also showed that both chemicals could alter steroidogenic pathway and increase estrogenicity. CHDM and TPA did not cause significant effects on proliferation rate of GH3 cells in the range of concentration tested. Given the extensive use of tritam™ and their loadings to the environment, their long-term environmental regulators and the environmentally relevant concentrations deserve further studies. Keywords: Early life stage test, H295R cell, GH3 cell, Tritan Acknowledgement – This study was supported by National Research Foundation of Korea (NRF, Project no. 2013R1A1A01016684).

MOI22

Effects of Copper on the Serum Parameters of Freshwater Fish (Oreochromis niloticus) in Differing Calcium Levels M. Canli, Cukurova University / Biology; E. G. CANLI, University of Cukurova / Biology

One of the most important factors affecting metal toxicity in the aquatic environment is the free ion levels of waters. Calcium (Ca²⁺) is the predominant ion in freshwater, and the free ion concentration of Ca²⁺ in the freshwater fish Oreochromis niloticus were exposed to copper in differing Ca²⁺ levels (15, 30 and 90 mg/L) using acute (0.3 μM, 72 h) and chronic (0.03 μM, 30 d) exposure protocols. Following the exposures, serum parameters (ALP, ALT, AST, protein, glucose, cholesterol, triglyceride, Na⁺, K⁺, Ca²⁺, Cl⁻) of fish were measured. Data showed that there was no significant alteration (P>0.05) in any of the studied parameters in control fish (in which only Cu²⁺ was added). However, there were several alterations following copper exposure, especially at the lowest Ca²⁺ level. Activities of ALP, ALT and AST decreased significantly at the lower Ca²⁺ levels in chronic exposure. Protein levels did not differ significantly in any of the exposure conditions. However, Cu exposure at the lowest Ca²⁺ level sharply increased the levels of glucose in the acute exposure, while there was no significant difference in the chronic exposure. Cholesterol levels decreased only at the lower Ca²⁺ levels in chronic exposure, but not in acute exposure. Similarly, triglyceride levels only altered at the lowest Ca²⁺ level both in acute and chronic exposures. In conclusion, this study showed that copper exposure of fish at lower Ca²⁺ levels caused more toxicities, comparing to the higher Ca²⁺ levels. Data suggest that Ca²⁺ levels of waters may be used in the evaluation of metal toxicity data from different waters with different chemical characteristics. Keywords: Copper, Calcium, Serum, Fish

MOI23

Cytotoxicity of herbicide clomazone and two formulations of this substance on rainbow trout gills cell line A. Feticolic, K. Hilscherova, Masaryk University, Faculty of Science. RECETOX / RECETOX Research Centre for Toxic Compounds in the Environment; M. Michelova, Masaryk University, RECETOX / Faculty of Science RECETOX; L. Blahova, Research Center for Toxic Compounds in Environment; D. Brícik, Institute for Pesticides and Environmental Protection; S. Gasic, Institute of Pesticides and Environmental Protection Chemistry and Environmental Sciences; D. Schlenk, University of California-Riverside / Department of Environmental Sciences

Diuron is one of the most commonly used herbicide in sugarcane cultivation in the Sao Paulo State. The risks that this compound represent for aquatic organisms that inhabit rivers and lagoons close to sugarcane crops are tough not well understood, despite it have been found in rivers and also subterranean water. Although diuron have a relative low persistence in the environment, its biodegradation metabolites, such as dichloroaniline (DCA), dichlorotoluene (DCTU) and dichlorophenyl-methyl-urea (DCPMU) could also represent a risk for the exposed biota. Moreover, commercial formulations of diuron generally present adjuvants that could further alter the effects and fate of diuron in fishes, despite all these aspects were not studied yet in these organisms. Considering the extensive use of diuron in sugarcane cultivation in the Sao Paulo State and the potential risks that this compound and its derived metabolites and adjuvants could pose to fish, in this work we measured a series of biochemical parameters in liver, gills and blood of Nile tilapias, in order to evaluate the negative effects that these compounds might exert in fish, at environmentally relevant concentrations. A series of experiments were done by exposing Nile tilapias to Diuron, DCA, DCPU and two alkylphenol surfactants commonly used as dispersants in pesticide formulations, octilphenol (OP) and nonylphenol (NP), alone or mixed, for seven days. Our results showed that both diuron and its biodegradation metabolites are prone to alter antioxidant defense systems and biotransformation enzymes, and were in some cases genotoxic to fish, changing DNA damage levels. Mixtures of Diuron with its metabolites caused slight differences in biomarkers responses compared to the effects of is in which only Cu²⁺ was added. However, there were several alterations following copper exposure, especially at the lowest Ca²⁺ level. Activities of ALP, ALT and AST decreased significantly at the lower Ca²⁺ levels in chronic exposure. Protein levels did not differ significantly in any of the exposure conditions. However, Cu exposure at the lowest Ca²⁺ level sharply increased the levels of glucose in the acute exposure, while there was no significant difference in the chronic exposure. Cholesterol levels decreased only at the lower Ca²⁺ levels in chronic exposure, but not in acute exposure. Similarly, triglyceride levels only altered at the lowest Ca²⁺ level both in acute and chronic exposures. In conclusion, this study showed that copper exposure of fish at lower Ca²⁺ levels caused more toxicities, comparing to the higher Ca²⁺ levels. Data suggest that Ca²⁺ levels of waters may be used in the evaluation of metal toxicity data from different waters with different chemical characteristics. Keywords: Copper, Calcium, Serum, Fish
MO124 Chemical treatment to reduce sugarcane vinasse toxicity: mortality and micronucleus decrease in Oreochromis niloticus (Pisces)

J.E. Correia, Universidade Estadual Paulista Julio de Mesquita Filho / Environmental Mutagenesis; C.A. Christofoletti, Fundacao Herminio Ometto / Environmental Mutagenesis and Reproduction

Background: Sugarcane vinasse is a by-product of ethanol production. Chemical treatment of these residue through pH adjustment with lime (CaOH). For toxicity analysis were used micronucleus test in erythrocytes, and arsenobetaine has shown that it is not metabolized by zebrafish. In this study, we investigated the effects of chemical treatment on the viability of Oreochromis niloticus exposed to sugarcane vinasse.

Methods: Freshwater fish species Cyprinus carpio was exposed to sugarcane vinasse at different pH levels. The exposure period was 24 hours. The results were analyzed by the Shapiro-Wilk test methods and Krukal-Wallis.

Results: The highest decrease in the micronucleus count was observed in the group treated with pH 7.0. The difference was statistically significant (p < 0.05).

Conclusions: Chemical treatment of sugarcane vinasse can reduce its toxicity to fish, reducing the risk of genotoxic effects. Further studies are needed to evaluate the impact of pH adjustment on other aquatic organisms and ecosystems.
effect. Plasma analysis via ELISA found significant Vtg induction in mature male fathead minnows (FHm) exposed to the E2 positive control. In contrast, only minimal Vtg induction was found in male LMB at any exposure age. This disparity in response between the two species to aqueous estrogenic stimuli was further explored by measuring endogenous FHM and mixed-gender juvenile (3 mph) LMB to a series of E2 treatments (~ 2.0 ng/L, 10 ng/L and 40 ng/L) with groups (n = 8) subsequent exposure to 10- and 14-d and plasma collected for Vtg analysis. Resulting response surfaces indicate a dose-response in FHm Vtg induction as a function of both duration of exposure and E2 concentration. In contrast, LMB produced minimal Vtg at all sample durations and exposure levels. Despite frequent occurrence of TO and detection of Vtg in native male LMB, this species may be poorly suited as a model for investigations into endocrine disruption under standard laboratory exposure scenarios.

MO128 Trophic transfer of the neurotoxin BMAA from the bivalve Dreissena polymorpha to the fish Rutilus rutilus and impact on the fish


Due to eutrophication of freshwater systems, the frequency of cyanobacteria proliferations is increasing worldwide and the presence of associated cyanotoxins represent a threat for ecosystems and for human health. Most cyanobacteria produce a neurotoxic amino acid β-methylamino-l-alanine (BMAA) linked to serious neurodegenerative diseases (amyotrophic lateral sclerosis (ALS), Alzheimer and Parkinson disease). Knowledge should be extended to the scale of BMAA accumulation in the foodweb and also about its impact on aquatic organisms. We previously demonstrated that the bivalve Dreissena polymorpha accumulated free and bound BMAA in tissues during an exposure to dissolved BMAA. Therefore D. polymorpha is potentially a BMAA-vector through the food web. This study focuses on the trophic transfer of BMAA between D.polymorpha and the fish Rutilus rutilus and the consequences on this latter in terms of: 1) BMAA accumulation and elimination in the liver, brain and muscle, 2) acetylcholinesterase (AChE) activity in the brain and muscle, 3) oxidative stress response in the liver via activities of enzymes catalase (CAT), superoxide dismutase (SOD), Elfoxyresorufin-O-deethylase (EROD), glutathione peroxidase (GPx), glutathione (GSH) and glutathione-S-transferase (GST), and 4) activity of digestive enzymes (amylase and trypsin) in the gut. Fish were fed with BMAA-toxicated mussels during a 2-weeks intoxication period followed by 1 week of depuration. Results show a BMAA trophic transfer from the bivalve to the fish with an accumulation of BMAA in the brain, liver and muscle of fish after 4 days of ingestion of BMAA-toxicated mussels. BMAA content in fish organs increased from day 4 to day 14 of depuration with maximum of 3.16 ± 0.07 µg BMAA g⁻¹ protein in muscle. BMAA content decreased during the depuration period in the fish liver and in the brain but increased in muscle up to 5.7 ± 4.35 µg BMAA g⁻¹ protein at the day 7 of depuration. No significant alteration in the activity of amylase, trypsin, SOD, GSH and GST enzymes was reported. In the liver GPx and EROD activity were respectively decreased and increased after 4 days of exposure, whereas CAT activity was decreased after 14 days. The neurotransmitter AChE showed a decreased activity in the brain at the end of the depuration period.

MO129 A preliminary exploration of the relationship between LC50 and individual body weight for fishes

C. Lu, Beijing Normal University / Beijing Normal University Academy of Disaster Reduction and Emergency Management

Prediction of lethal effect on organism of interest that lacks of test data is critical for risk assessment of chemicals on aquatic ecosystems. The authors considered body weight of organism plays an important role in counteracting toxic stress imposed by certain chemical. To explore the influence of body weight to toxic effects, the authors considered body weight of organism plays an important role in counteracting toxic stress imposed by certain chemical. To explore the influence of body weight to toxic effects, the authors considered body weight (LC50, Lead, Zinc).

MO130 Effect of dietary 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) exposure in developing medaka fish (Oryzias latipes). M. Gonzalez-Donde, National Institute for Agricultural and Food Research and Technology / Department of Environment; S. J. Karjalainen, University of Jyväskyla / Biological and Environmental Science; E. Vehnäniemi, University of Jyväskyla / Department of Biological and Environmental Science; A. Väisänen, University of Jyväskyla / Department of Chemistry; A. Karjalainen, ECHA-European Chemicals Agency

Metal mining can have significant and long lasting effects on aquatic environment. Recently, the focus has turned towards the excessive loads of natural substances caused by metal mining. Elevated manganese (Mn) and sulfate (SO₄) concentrations have been observed in water bodies receiving metal mining effluents, and at worst SO₄ induced salinization has led to halocline development, and thus hypoxia or anoxia in the hypolimnion of the recipient lakes. However, the
effects of such natural substances on aquatic organisms are not well known. We studied the effects of manganese sulfate (MnSO₄) on whitefish (Coregonus lavaretus L) embryos and larvae, and the significance of parental effect on observed responses. Early development in fish is considered the most sensitive phase to environmental stressors, with fertilization and hatching the most sensitive stages of the early development. Metal exposure during any stage of early development may affect the larval motility and larvae. Also, abnormally elevated salinity can delay the survival of fertilized fish embryos throughout their development. Whitefish embryos and larvae were exposed to eight ecologically relevant MnSO₄ concentrations in geometric series from 0.007 to 568.4 mg L⁻¹ of SO₄ and 0.004 to 325.0 mg L⁻¹ of Mn under semi-static conditions. The continuous exposure was started from the fertilization, and larvae were reared for four or five days after hatching. Embryonic motility was observed 2-3 times per week and number of hatchet and dead larvae were observed daily. Embryo samples for metal uptake and gene expression analyses were collected before the water temperature rising was initiated to induce hatching. The MnSO₄ exposure did not have clear effect on the total fertilization success, but it decreased the total survival of embryos and larvae. None of the embryo hatched successfully in the highest MnSO₄ concentration (568.4 mg SO₄ L⁻¹, 325.0 mg Mn L⁻¹), and hatching and survival of the larvae was significantly decreased in the second highest concentration (113.7 mg SO₄ L⁻¹, 65.0 mg Mn L⁻¹) as well. Even though the effects on survival were most evident in the highest concentrations, the preliminary embryonic metal uptake results showed elevated Mn levels in embryos in nearly all exposure concentrations. The ongoing gene expression analyses of the embryos may further permit to get an accurate picture. The role of parental effect on the observed responses will also be defined.

MO133

Toward identification of fish specific estrogens through effect-directed analysis based on zebrafish in vitro and in vivo reporter gene assays

B. Nave, F. Van der Graaf, I. van der Graaf, Grebiński, R. Borst, S. Brién, INERIS / Ecotoxicology Unit; N. Creusot, C. Turès, INERIS; J. Porcher, INERIS / UMR SEBIO; S. Ait-Assia, INERIS / Ecotoxicology Unit

Estrogens are present in all vertebrates, from teleosts to mammals, which can bind to and activate estrogen receptors (ERs) through a conserved mechanism. However, existence of cross-species differences as regards ER subtypes and the binding affinity of (xeno)estrogens on ERs, as well as the cellular context may lead to differential ER activation by environmental contaminants when assessing hazard on aquatic organisms. For this purpose, we previously developed, characterized and demonstrated the functionality of stable zebrafish (zf)-ER subtypes (zfERα, zfERβ1, zfERβ2) in the zebrafish liver (ZFL) cell line and transgenic zebrafish (zf-dXRE-EGFP) expressing estrogenic activity in complex mixtures. In the present study, we used zf-zebrafish (based in vitro and in vivo bioassays) to assess estrogenic activity in surface water by investigating 20 French river sites using polar organic chemical integrative sampling (POCIS). Simultaneously, the human MELN bioassay (MCF-7 cells-based, expressing hERα) was used to study cross-species differences. POCIS-based bio-monitoring provide significant information in the estrogenic activities at the organism level, which were observed in embryo assay at sites that were highly active on in vitro bioassays. These in vivo results are relevant for hazard assessment as they reveal that detected active compounds target radial glial cells expressing brain aromatase which are known to be involved in neurogenesis. In addition, combining these zf-based tools within an EDA approach, we were able to isolate fractions that were active only on zfERβ2 subtype, hence reinforcing the hypothesis of species-specificity for, yet unknown, ER ligands. Further investigation using mass spectrometry techniques is foreseen to identify fish-specific active compounds which will allow improving bioassay-based environmental risk assessment towards aquatic species.

MO134

The effect of five quaternary ammonium ionic liquids on Danio rerio embryos: do they cause oxidative stress?

A. Jakubowska, Silesian University of Technology / Environmental Biotechnology Department; E. Kuster, Helmholtz Centre for Environmental Research (UFZ); E. Grabińska-Sota, Silesian University of Technology / Environmental Biotechnology Faculty

Amongst many industrial applications quaternary ammonium ionic liquids (QAILs) are being tested as novel pesticides. They were proven very effective against many fungi and bacteria strains, as well as insects. Nevertheless they can constitute a threat towards non-target organisms after introducing to the market. They can reach the environment with factory effluents, waste water treatment plants effluents, accidental spills, as well as after being washed out of the site of application into the ground or surface waters. QAILs with didecylmethylammonium cations were already detected in different environmental compartments. The toxic mode of ionic liquids’ action is still not well described. The molecular target of ionic liquids with long alkyl chains are membranes but also oxidative stress is thought as a mode of action. We aimed to evaluate toxic effects of five QAILs towards zebrafish (Danio rerio) embryos both on the levels of lethal and oxidative stress in search of correlation with toxicity. Depending on the molecule the formulas involved one, two or three didecylmethylammonium cations (IDDA) and nitrite, nitrate or citrate anions. To assess the toxicity of the five compounds the standard 96 h fish embryo acute toxicity (FET) test was performed according to the OECD 236 Guideline. We also measured the relative oxygen consumption in zebrafish by using 2,7’-dichlorodihydrorhodamine diacetate (DCPH-DA) after treating them with the most toxic compound in concentrations of LC₅₀, LC₃₀, LC₁₀ and LC₅ for 6 and 24 h. Tet-butyl hydroperoxide (TBHP), a well-known prooxidant was used as a reference compound. The fluorescence proportional to the level of ROS was measured. Five QAILs are highly toxic to Danio rerio embryos. The obtained LC₅₀ values were found in the low µg/L range. Their lethal action is rapid and occurs before 24 h of the test duration. Hence, the lethal effects were expressed as 24 h LC₅₀. The toxicity highly depends on the number of [DDA] cations and is not correlated with the anion type. The most toxic compound can cause oxidative stress after 24 h of exposure to low effect concentrations.

MO135

Mechanism of PFOS effects on posterior swim bladder chamber inflation in zebrafish larvae

L. Vergauwen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; A. Hagaens, Zebrafishlab Dept Veterinary Sciences; E. Stinckens, Zebrafishlab Veterinary Physiology and Biochemistry Department of Veterinary Sciences; L. Beton, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; N. Vinas, Mississippi State University; D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences

Perfluorooctane sulfonate (PFOS) is one of the most commonly detected perfluorinated alkylated substances in the aquatic environment due to its persistence and the degradation of less stable compounds to PFOS. PFOS is known to cause developmental effects in fish. The main effect of PFOS in zebrafish larva is to cause an inflated swim bladder. As no previous studies have focused on the effect of PFOS on zebrafish swim bladder inflation, the exact mechanisms leading to this effect are currently unknown. The objective of this study was to investigate the mechanism by which PFOS impairs swim bladder inflation. To this end, we first determined the exposure windows during early zebrafish development that are sensitive to PFOS exposure and result in impaired swim bladder inflation. Seven different time windows of exposure (1–48, 1–72, 1–120, 1–144, 48–144, 72–144, 120–144 h post fertilization (hpf)) were tested based on the different developmental stages of the swim bladder. These seven time windows were tested for four concentrations corresponding to the EC-values of 1, 10, 80 and 95% impaired swim bladder inflation (EC₁₀ = 0.70 mg L⁻¹, EC₁₀ = 1.14 mg L⁻¹, EC₁₀ = 3.07 mg L⁻¹ and EC₁₀ = 13.9 mg L⁻¹). At EC₁₀, no significant effects were found for the tested parameters while 1.14 mg L⁻¹ (resulted in a reduction of larval length. For 3.07 and 4.28 mg L⁻¹, the number of larvae affected and the severity of effects caused by PFOS were dependent on the time window of exposure. Larvae that were only exposed early (1–48 hpf) or late (120–144 hpf) hpf died. However, those exposed for 60 hpf were able to develop to the corresponding apical endpoint. The results demonstrate that PFOS does not affect the budding phase, and does not cause deflation of already inflated swim bladders. PFOS clearly affects processes that take place during the inflation phase and might also have an effect on the formation of the tissue layers forming the swim bladder. Currently, we are further investigating these processes by performing microarray analyses and relating our findings to accumulated doses.

MO136

Evaluation of the Adverse Outcome Pathway (AOP) of aromatase inhibition from the molecular initiating event to the adverse outcome affecting the sexual maturation of zebrafish


Adverse outcome pathways (AOPs) describe the linkage of effects of environmental hazards on the molecular level, based on their mode-of-action, to their outcome on organismal/population level. AOPs are intended to provide information on molecular as well as apical level and thus can be considered as useful tool for interpretation of results from ecotoxicological studies. However, it is not always possible to develop a complete AOP for any analyses due to lack of a priori knowledge. An AOP can therefore be defined as an unequivocal link to the corresponding apical endpoint. To date, most studies either focus on the apical endpoints of standard tests or the evaluation of effects at the molecular level. Combined studies are short-handed. We here present the data of a proof-of-principle study with the non-steroidal aromatase inhibitor fadrozole (test concentrations: 10 µg/L, 32 µg/L, 100 µg/L) in a Fish Sexual Development Test (FSDT) with zebrafish (Danio rerio) in compliance with the OECD TG 234 and in combination with gene expression analyses at different time points (48 hpf, 96 hpf, 28 dpf, 96 dpf). The purpose was to define molecular endpoints altered by aromatase treatment and resulting in adverse apical effects. Observed apical effects of fadrozole during the sexual development of fish include a complete shift of the
sex ratio towards males and accelerated maturation already at low concentrations (10 µg/L fadrozole). This is due to the mode of action of fadrozole, which inhibits the activity of Cyp19a1 (aromatase), leading to an inhibition of the conversion of C19-androgens to C18-estrogens, and thus, modulating the steroid hormone concentrations controlling the sex ratio. A molecular key event is the regulation of genes possessing an estrogen-responsive element (ERE) in their promoter region. According to this assumption, we found a significant down-regulation of vtg1 at the mRNA level in treatments compared to the control as early as 48 hpf and 96 hpf. Further, a regulation of cyp14a1b mRNA was observed at 63 dpf, a gene also possessing an ERE. Other regulated genes cumulate in the steroid and terpenoid synthesis pathway (e.g. mvd, lss, scnn1d) and in lipid transport/metabolic processes (star, apoA1, vtg1), pathways likely to be controlled by endocrine mechanisms. Taken together, we were able to identify molecular as well as apical endpoints affected by fadrozole and provide evidence for a more comprehensive AOP of aromatase inhibition during sexual development of fish in a single study.

**Systems Biology: increasing the capacity, understanding ecosystems and response mechanisms to model chemicals (including nanomaterials) (P)**

**MO137**

**Sorting the signals: meta-analysis of transcriptome data of zebrafish embryos after chemical exposure**

A. Schmetter, Department of Bioanalytical Ecotoxicology; R. Altmann, UFR CNRS Centre for Environmental Research / Department of Bioanalytical Ecotoxicology; W. Busch, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology

Adverse effects of chemicals on cells and organisms are in many cases either preceded by or result in gene expression changes. This makes transcription analysis a promising tool in ecotoxicological research, e.g. as future bioassay or diagnostic tool for biomonitoring. There are several advantages in comparison to established tools, e.g. the non-targeted approach, allowing assessment of several modes-of-action at the same time, or the possibility to combine assessment of adverse effects with identification of effect-drivers, which will also be helpful in the establishment of adverse outcome pathways (AOPs). Several studies have found specific gene-expression changes in zebrafish embryos after exposure to single chemicals. This shows that the zebrafish embryo – already an established model organism in ecotoxicology – might also have the potential to become an *in vivo* model for ecotoxicogenomics. To take a step from proof-of-principle studies towards usability of transcriptome analysis in biomonitoring or toxicity assessments of chemicals, a systematic and comparative insight into expression signatures of chemical exposure seems promising. We therefore perform a meta-analysis of already published transcriptome data. The analysis will combine the data of several studies using different chemicals, concentrations and timepoints. The aim is to get an overview of commonly or specifically affected genes and pathways. This will hopefully allow the separation of general stress answers and developmental disturbances from specific effects of chemicals. Additionally, specific focus will be put on the influence of factors like chemicals, concentration, exposure time and duration or array design on the gene-expression pattern. The results of the meta-analysis will also help to build hypotheses on toxicity of mixtures on a molecular level.

**MO138**

**Metabolic profiling of chicken embryos exposed to PFOA or to PPARα and PPARγ activators**

A. Mattsson, Uppsala University / Dept of Environmental Toxicology; A. Kärman, Orebro University / SCHOOL OF SCIENCE AND TECHNOLOGY; B. Brunstrom, University of Uppsala / Dept of Environmental Toxicology

Metabolomics (or metabonomics) is the profiling of hundreds or thousands of metabolites in a tissue or body fluid and how it changes in response to, for instance, treatment with chemicals. Avian embryos develop in the eggs separately from their mother. Thus the embryonated chicken egg provides a convenient model for exploring effects of compounds on embryonic development without confounding maternal factors. The allantois is an extra embryonic membrane that collects urine and metabolic waste and serves as a reservoir for nutrients. Blood and allantoic fluid can easily be sampled for metabolic profiling. The metabolic profile in the allantoic fluid likely reflects changes over a longer period while blood plasma gives a more instant picture of the metabolome. The overall objective of this study was to explore the chicken egg as a model system for studying pollutant-induced changes in the metabolic profile of the developing embryo. Perfluorooctanoic acid (PFOA) is a known toxic environmental contaminant and its effects as an endocrine disruptor can be mediated by peroxisome proliferator-activated receptors (PPARs), which are lipid sensors and regulate energy metabolism and tissue differentiation. We tested the hypothesis that PFOA induces metabolic changes similar to those induced by PPARα- and/or PPARγ-activation. Chicken embryos were exposed via egg injection on embryonic day 7 (E7) to 50 or 250 µg PFOA/egg or to 150 µg/egg of GW7647 (PPARα-agonist) or rosiglitazone (PPARγ-agonist). Blood plasma and allantoic fluid were collected on E12 and metabolite concentrations were analyzed using GC/MS and LC/MS. Multivariate data analysis of allantochlor fluid revealed drastic changes in metabolic profiles by GW7647 and rosiglitazone; these groups formed two completely separate clusters in OPLS-DA plots. In plasma, only GW7647 separated significantly from the controls. Concentrations of only a few metabolites were significantly changed by PFOA and these were not affected by the PPAR-agonists. The injected doses of 50 and 250 µg PFOA/egg resulted in liver concentrations of 1 and 4 µg PFOA/g, respectively. Thus, in spite of internal PFOA doses exceeding those in the control, some wild birds we found only little effects on metabolite concentrations. Our results suggest that both allantoic fluid and plasma are useful matrixes for analyzing metabolic perturbations in the chicken embryo. We found no evidence from the metabolic profile that PFOA induces PPARs in chicken embryos.

**MO139**

**Effects of diameze pregnate on biochemical blood parameters in Wistar rats**

F. Campos Pereira, R. Vaz Hara, Uneps - Institute of Biology / Biology; L.C. Gonçalves, L. Souza Franco, R. Ferraz Curtolo, G.A. Alves, G.D. Severe-Aguiar, Uniarias / Programa de PósGraduação em Ciências Biomédicas; M.A. Marín-Morales, UNIVERSIDADE ESTADUAL PAULISTA - UNESP / Department of Biology

Cemeteries have been emerged as a major source of soil and groundwater contamination due to percolation of the necrochurume resulting from cadaveric putrefaction. This compound is rich in several chemical substances, among them the diameze pregnate (C₁₂H₂₈N₄), a polyanime that plays an important physiological role in the formation of homocystine, amidotransferase, and to participate in DNA synthesis and replication, and RNA transcription. Recently, it have been reported that human exposure to necrochurume could lead to health risks. This study has evaluated biochemical parameters of blood of animals submitted to different concentration of diameze pregnate. Four experimental groups were conducted: Control group (Co- distilled water), group 1 (1 g/kg body weight), group 2 (5 g/kg body weight), and group 3 (10 g/kg body weight). The necrochurume was administrated orally (gavage), and the blood was collected 15, 30 and 56 days after the treatment. The samples were centrifuged at 2,500 xg, in order to obtain the plasma. The plasmatic levels of glucose, total cholesterol, HDL and triglycerides were determined with commercial kits Laborních®. Biochemical analyses were performed by spectrophotometry, using the Asys Expert Plus Microplate reader, and the significance analysis of the results was performed by ANOVA (test/Tukey, p < 0.05). The blood levels of glucose, total cholesterol, HDL and triglycerides were evaluated in the control groups in three distinct periods. The levels of total cholesterol, HDL and triglycerides showed a significant decrease in the animals treated for 15 days with the lowest concentration. There was a significant decrease in glucose blood levels at all concentrations tested for 30 days. The other results showed no significant differences for any of the parameters. Based on the present work, we highlight the importance of developing further studies with this substance, since it was able to induce a hypoglycemic state and reduce the levels of non-esterified fatty acids and total lipids. These results suggest that increasing blood levels of pregnate may induce disorders in the physiological regulation of triglycerides and the connection between cholesterol and lipid metabolism. Additionally, our data might indicate that around 56 days of treatment, the organism may be presenting a compensatory biochemical response for recovering homeostasis.

**MO140**

**Effect of ethanol, methanol or dimethyl sulfoxide on cytochrome P450IA activity**

S. Sakalli, Faculty of Fisheries and Protection of Waters; V. Burkina, University of South Boehmia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocences Vodnany Czech Republic; V. Zlabek, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters LECIB; G. Zamaratskaia, Swedish University of Agricultural Sciences / Department of Food Science

The impact of three organic solvents on cytochrome P450 (CYP) 1A enzyme activity was investigated in hepatic microsomes of rainbow trout. Liver samples containing nine fish fingers were used for in vitro the CYP1A-mediated formation of resorufin from 7-ethoxyresorufin (EROD) was measured in the presence of methanol, ethanol and dimethyl sulfoxide (DMSO) at concentrations from 0.01 to 10 mg/mL. The values of the EROD activity when ethanol, methanol or DMSO at the concentrations above 0.5% was added. In the presence of 3% of ethanol, EROD activity was completely blocked, as suggested by undetectable formation of resorufin. No changes in EROD activity were observed in the presence of organic solvents at concentrations below 0.5%. To the best of our knowledge, this is the first study to show a strong in vitro effect of organic solvents on EROD activity in the microsomes from rainbow trout. These findings should be considered when designing in vitro metabolic studies with fish hepatic microsomes. Unlikely, the concentration of these organic solvents, which might affect CYP1A activity, for solubilisation of substrates and inhibitors in in vitro microsomal...
studies should be avoided. Further studies are needed to identify organic solvents with no or minimal effect on CYP450 activity. Such studies should involve all commonly used probe reactions that represent activity of specific CYP450 isofoms. Keywords: Organic solvents; 7-ethoxresorufin-O-deethylase; In vitro model; rainbow trout. MO143 A systems biological approach to understanding narcosis using C. elegans and RT cell lines E.K. Brockmeier, University of Liverpool / Functional and Comparative Genomics; D. Basili, University of Liverpool / Institute of Integrative Biology; G. Hodges, Unilever Research / Safety and Environmental Assurance Centre; E. Butler, Cranfield University; S. Gutsell, Unilever / SEAC; F. Falciani, University of Liverpool / Functional Genomics Narcotic chemicals act on fundamental cellular functions which can impact a wide range of species when they enter into the environment. These chemicals represent a large volume and number of the chemicals used in industrial applications. Currently there is no mechanistic understanding of narcosis and how this baseline toxicity occurs on a molecular level. The objective of this project is to use the RT gill cell line and Caenorhabditis elegans to develop an advance outcome pathway (AOP) for narcosis which will be relevant across multiple species. For this project, LC50 data was generated from both C. elegans and the RT gill cell line from a panel of 30 chemicals, which include 15 proposed non-polar narcotics and 15 proposed polar narcotics. Based on regression correlations with the existing USEPA LC50 data for fathead minnow (Pimephales promelas), these predicted narcotics appear to act via a narcosis mechanism albeit for the nematodal chemical 2-hydroxyethyl ether. For mammalian endpoints, C. elegans and C. elegans to 1/10 of the LC50 for 24 hours and extracted RNA for microarray analysis. From this data set we are able to determine the impacts of narcotic exposure on this organism as well as to depict the differences in molecular responses between polar and non-polar narcotics. Using the SeahorseBioscience platform, which utilizes fluorescent probes for in vitro testing of oxygen consumption, we are able to correlate these molecular responses and LC50 data to concentrations and physiological effects. All in vitro data from the RT gill cell line provides a complement to these results and enables the comparison of nematode to aquatic animal responses without the need for animal testing. These data will be coupled with metabolomics data sets in order to develop an advance outcome pathway for narcosis toxicity for comparison with proposed existing fish based narcosis AOPs. They will also be linked with parallel studies to be conducted in Daphnia magna to better understand the impacts of this class of chemical across multiple species. Through this project we will gain a better understanding using a systems biological approach of the toxic mechanism of action of this expansive class of environmental toxicants.

MO144 Toxicological studies of polymeric nanoparticles containing essential oil of neen L.F. Fraceto, Sao Paulo State University - UNESP / Environmental Chemistry; T. Pasquoto, Universidade Federal de São Carlos; A. Gonzalez, São Paulo State University - UNESP; E.V. Campos, Sao Paulo State University - UNESP / Engenharia Ambiental; J. de Oliveira, Sao Paulo State University - UNESP; R. de Lima, Universidade de Sorocaba Neen (Azadirachta indica) is a tree belonging to the Meliaceae family. Azadirachtin, a tetranortriterpenoid, is the major active ingredient isolated from neen, which is known to disrupt the metamorphosis of insects. However, the major problem is its sensitivity to photodegradation so it is rapidly lost when exposed to sunlight, also because of its low volatility and solubility, it is difficult to use in an aqueous system. In this study, we investigated the toxicological and biological effects of nanoparticles containing neen oil. Polymeric nanocapsules were able to reduce cell viability in HeLa cancer cell type. The MTT assay results nanoparticles loading neen oil showed good colloidal parameters. All nanocapsules loading neen oil showed good in vitro cell viability. The results of these studies conducted in Allium cepa showed that in a 4% solution of neen oil against 1/10 of the LC50 was performed according to the method of water/oil emulsion and evaporation of the solvent. The stability of nanoparticles formulations (with and without neen oil) was investigated by the measurements of some physico-chemical properties such as mean size diameter, polydispersivity, zeta potential and encapsulation efficiency as function of time (120 days). The toxicological assays were done through cytotoxic assays (MTT and Cytometer Image) and genotoxic assays (comet assay and chromosomal aberrations using Allium cepa). The results showed that nanocapsules loading neen oil showed good colloid parameters. All prepared nanoparticles presented polydispersivity lower than 0.2 showed good homogeneity of the formulation, zeta potential around -27 mV. The encapsulation efficiency of neen oil in nanoparticles with and without with high genotoxicity. The results showed that in the T3T cells the nanoparticles were not cytotoxicity. All nanoparticles were able to reduce cell viability in HiLa cancer cell type. The MTT assays showed that nanoparticles with and without neen oil were cytotoxic to human lymphocytes. According to the Allium cepa results nanoparticles loading neen oil did not alter the mitotic index and the damages have not index significant. These preliminary results indicate that the encapsulation of neen oil in polymeric nanoparticles could be an interesting alternative to control pests in agriculture, however, the toxicological results indicate that these nanoparticles could be toxic to non-target organisms. Acknowledgments: Fapesp, Fundunpec, CNPq and CAPES.
Identification toxic pathways of graphene nanomaterials in the nematode, Caenorhabditis elegans using integrated multi-OMICS approach

J. Yang, N. Chatterjee, University of Seoul; S. Kim, Pusan National University; J. Hong, Kyung Hee University; C.P. Roca, Universitat Rovira i Virgili / Department of Chemical Engineering; F. Giralt, Universitat Rovira i Virgili; I. Chui, School of Environmental Engineering Graduate School of Energy and Environment System Engineering, University of Hong Kong.

Graphene and its derivatives have attracted great research interest for their potential applications in electronics, energy, materials and biomedical areas. However, little information of their toxicity and bio-compatibility is available. The system toxicity approach using 'multi-OMICS-profiling-techniques' (transcriptomics, proteomics and metabolomics) has proven to be a powerful tool for unraveling complex machinery underlying various physiological, and pathophysiological processes, and they have been successfully utilized in various fields, including stress biology and toxicology. The integration of these OMICS technologies has the potential to reveal a much more detailed view of homeostasis and regulatory networks than when used individually, which is particularly powerful for elucidating potential mechanisms of toxicity of new chemicals, such as graphene nanomaterials. In this study, using an integrated multi-OMICS approach, potential mechanisms of toxicity of graphene nanomaterials were investigated in the nematode Caenorhabditis elegans. We first screened the toxic potential and uptake of five different graphene nanomaterials (three kind of graphene nanopl a telets –pristine, amine and carboxylated and single layered graphene and few layer graphene) in the nematode C. elegans. Further, we found that graphene oxide led to a significant decrease of reproduction. Then, we selected graphene oxide for further in-depth multi-OMICS study to elucidate the underlying mechanism of reproductive toxicity. Microarray followed by pathway analysis was conducted on C. elegans exposed to graphene oxide. Non-targeted NMR based metabolicomic as well as targeted GC-MS based lipidomics, were subsequently conducted. Multi-OMICS data were analyzed by Meta/Analyst and were also conducted to identify altered pathways by graphene oxide. Several pathways related to reproduction, such as WNT and MAPK, emerged from the integrated OMICS analyses. We tried to confirm the link between these pathways and reproduction decline by using the C. elegans functional genomics tool. Loss-of-function mutants of genes from these pathways were exposed to graphene oxide and their responses were compared with that of wildtype. Overall results suggest that integrated multi-OMICS approaches provide comprehensive insights of the toxicity of graphene oxide, which will guide further mechanism study.

Assessing the effects of silver nanoparticles in Caenorhabditis elegans: adverse effects, and possible mechanisms of toxicity

P. Samuttrai, D.A. Drynda, T.F. Fernandes, Heriot-Watt University / School of Life Sciences

Given the rise in applications of nanotechnology to a wide range of industries, it is likely that release and few layer graphene will end up in the environment. However, the effects, as well as the, mechanistic pathways of toxicity of engineered nanomaterials in the environment are not thoroughly known. Therefore, it is important to investigate any effects induced by these nanomaterials. In this research, two silver nanomaterials (NM300K and NM302) from the European Commission’s Joint Research Centre (JRC), as well as the effects of a silver salt Ni(NO3)2 on their toxicity based on the nematode Caenorhabditis elegans. The effect of silver nanomaterials was assessed by studying lethality and reproduction of particular nematode strains with the absence in specific genes. In addition, the authors assessed whether oxidative stress was one of the major adverse effects, and possible mechanisms of toxicity

Toxicogenomic profile linked to higher level of the soil invertebrate Enchythraeus crypticus to Ni nanoparticles

S.L. Gomes, University of Aveiro / Department of Biology CESAM; J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience Terrestrial Ecology; M. Amorim, Universidade de Aveiro / Department of Biology and CESAM

The potential impact and the mechanisms of toxicity of Ni-Nanomaterials (Ni-NMs) on the invertebrates is still poorly understood, in particular in soil organisms. The understanding of the mechanisms of toxicity enabled by omic techniques may provide tools which enable the prediction of long-term effects, based on shorter term measures, thus improving the ability to predict risk of NMs. The aims of the present study are to understand the mechanisms of response (based on gene expression analysis) of the soil invertebrate Enchythraeus crypticus (Oligochaeta, Enchythraeidae) to Ni-NPs (in comparison with NiNO3) and to link the knowledge provided by gene expression analysis with effects at higher levels of biological organization (e.g. energy budgets, reproduction, survival). To investigate this, organisms were exposed to the reproduction-effect concentrations EC20 and EC50 of Ag NPs and EC20 and EC50 of Ag NMs on the invertebrates (defined for the test species). Results showed a clear differentiation between times of exposure, with a higher number of genes being affected after 3 days of exposure. Further, a clear separation between NPs and Ni salt was profiled, indicating specific mechanisms of response or different time events. The mechanistic information provided by gene expression analysis was integrated with effects at higher levels of biological organization in an Adaptive Outcome Pathway (AOP) format allowing a visualisation and integration of knowledge. Keywords: High-throughput, modes of action, nickel nanoparticles, soil
Titanium dioxide nanoparticles (NPs) have been widely applied in daily life. They are used, mainly in pigments, with 70% of its production volume applied in paints, plastics, inks, foods, and toothpastes. Due to its larger use in the industry and daily life, many TiO₂ residues are released into the environment, and currently, TiO₂ NPs are being considered an emerging environmental contaminant. The aim of this study was to question whether TiO₂-NPs with different size-range, crystal-line form could affect iron homeostasis with *Pseudomonas brassicacearum* a plant root-associated bacterium (Achouak et al. 2000). Under iron-limitation, *Escherichia coli* produces a small regulatory RNA (sRNA) RyhB, which functions to facilitate degradation of the mRNAs of several target genes encoding iron-utilizing enzymes, iron acquisition systems, and iron storage factors (Masse and Gottesman 2002). This phenomenon is now referred to as “an iron-sparing” response. In *Pseudomonas* species, regulation of iron homeostasis involves two (or more) sRNA named PrrF (for *Pseudomonas* regulatory RNA involving iron), which are functionally analogous to RyhB (Wilderman et al. 2004). Two PrrF sRNAs were identified in the genome of *P. brassicacearum* (Ortet et al. 2011), prrF1 and prrF2. To investigate the role of TiO₂ NPs in iron homeostasis with *P. brassicacearum*, we prepared two shapes of anatase titania particles, and determined their biological activity by analyzing TiO₂ NPs-bacterial interaction, their impact on iron content and prrF1 and prrF2 sRNA expression. It was essential to properly prepare and characterize nanoparticles before and after utilization in vitro. To overcome the analytical challenges associated to the tracing unlabelled TiO₂ NPs, we used hyperspectral microscopy and inductively coupled plasma mass spectrometry (ICP-MS). Overall, the results of the present study demonstrated that TiO₂ NPs did not show any impact on bacterial growth, however they altered the bacterial physiology as they induced an oxidant stress and altered iron homeostasis. Although we used two different crystalline forms that differ by the size and the shape, we noticed almost the same mechanism of toxicity towards bacteria. Our findings yield new insights into the mechanism underlying the TiO₂ NPs toxicity toward bacteria. The expression of prrF sRNA represented a good indicator of intracellular iron status and consequently of the mode of TiO₂ NPs action. 

### MO151
**TiO₂ Nanoparticles: evaluation of the cytotoxicity and genotoxicity and effects on soil microorganisms**

M. Pascoli, University of Sorocaba; T. Pasquoto, Federal University of Sao Carlos USFSCar Soroca / Biotechnology; D.T. Rheder, Unio / Biotechnology; E.V. Campos, Sao Paulo State University - UNESP / Engenharia Ambiental; C.R. Maruyama, Federal University of Sao Carlos USFSCar Soroca / Biotechnology; L.F. Fraçeto, Sao Paulo State University - UNESP / Environmental Chemistry; R.d. Lemes UFRJ/CPCT.

Among all metallic nanomaterials titanium Dioxide (NP-TiO₂) is one of the most worrying due to its high production and use in commercial goods. In both soil and water the nanoparticles can suffer alterations and interact with soil components, increasing its toxicity. Within this context, this work has investigated titanium dioxide nanoparticles 100% Anatase (Aldrich) and a mixture of 80% Anatase and 20% Rutile titania (Aeroxide) and its physicochemical alterations and their relation to the medium in which it disperses, as well as the toxicity. For the evaluation some molecular and feasibility analyses were performed (MIC and UFC counting) of soil microbiota, as well as analyses on the cytotoxicity and genotoxicity through Allium cepa analyses, Comet analyses, MITT and Image Citometry. The behavior of physicochemical titanium dioxide nanoparticles in different mediums (DMEN, McCoy, RPMI and water) in the presence or absence of UV light presented alterations, that reflected in some cytotoxicity and genotoxicity tests, once NP-TiO₂ (Anatase e Aerosolox) show different behavior depending on the medium they are dispersed. In Allium cepa tests the nanoparticles presented significant alterations in the concentrations tested (1, 10 e 100 µg/mL). The results reveal that the anatase at 1 and 10 µg/mL presented weaker values significantly different when compared to the control in all treatments. Regarding the differences found between the two types of nanoparticles we could observe that the results presented significant differences for concentrations at 10µg/mL without exposure to ultraviolet light, being the damages on Anatase nanoparticles stronger and at the concentration at 1 and 10µg/mL with exposure to ultraviolet light, being NP-TiO₂ (Anatase e Aerosolox) the studies performed with soil microbiota showed that over time some variation occurred in relation to the type of existing bacteria variation, which has also occurred among the different NP-TiO₂ tested. The physicochemical characterization proved to be essential for the best understanding of the biological effects of NPs. The contribution of the present work for the area of nanoparticles toxicity is of great value as it helps on the understanding of the behavior of these materials, what is essential for their use in a safe way, causing minimum damage to the environment and human beings.

### MO152
**Canola plant potentiates the response of a soil-microbes ecosystem to ceramic oxide nanomaterials**

M. Hamidat, Aix Marseille Université / Laboratory of Microbial Ecology of the Rhizosphere and Extreme Environments LEMiRE UMR CNRS CEA Aix Marseille University iCEINT CEREGE Extreme Environment CEDRE EUROPE dis de l’Aix en Provence France; M. Barakat, CNRS / Laboratory of Microbial Ecology of the Rhizosphere Extreme Environments LEMiRE UMR CNRS CEA Aix Marseille University iCEINT CEREGE EUROPE dis de l’Aix en Provence France; P. Orte, CEA Cadarache / Laboratory of Microbial Ecology of the Rhizosphere Extreme Environments LEMiRE UMR CNRS CEA Aix Marseille University iCEINT CEREGE EUROPE dis de l’Aix en Provence France; W. Achouak, UMR 7265 CNRS-CEA Aix Marseille Univ / LLaboratory of Microbial Ecology of the Rhizosphere Extreme Environments LEMiRE UMR CNRS CEA Aix Marseille University Cedaarache SaintPaulLezDurance iCEINT CEREGE Extreme Environment LEMiRE UMR CNRS CEA Aix Marseille University Cedaarache SaintPaulLezDurance iCEINT CEREGE EUROPE dis de l’Aix en Provence France; C SANTAELLA, CNRSCEAAix-Aix Marseille University / Plant Biology and Microbial Biotechnology

With a global production of 10,000 metric tons/year, CeO₂ nanomaterials have a wide scope of applications in industrial processes and in everyday consumer products. The dissemination of these nanoparticles raises environmental concerns especially for soils through the disposal of biosolids. Soil is a complex system that hosts a complex food web, which will include plants and bacteria. In the literature, there are already strong evidences that CeO₂ nanomaterials have deleterious effects on plants and on soil bacterial communities. Recently, Ge et al. 1 showed that nano-CeO₂ (0.1 g kg⁻¹) did not affect soil microbial communities, but altered soil bacterial communities in soybean planted soils, showing that plants interactively stimulate nano-CeO₂ effects on soil, possibly due to belowground C shifts since plant growth was impacted. Still, current knowledge on CeO₂ nanomaterials on soil-based ecosystem comes from exposure to concentration of NMs that are far from those predicted in soil (0.28 - 1.12 mg kg⁻¹) or even in biosolids (0.53–9.10 mg kg⁻¹). In order to focus on more realistic exposure, we have explored the response of canola (Brassica napus) to nano-CeO₂ and on soil bacterial communities in which it disperses, as well as the toxicity. For the evaluation some molecular and feasibility analyses were performed (MIC and UFC counting) of soil microbiota, as well as analyses on the cytotoxicity and genotoxicity through Allium cepa analyses, Comet analyses, MITT and Image Citometry. The behavior of physicochemical titanium dioxide nanoparticles in different mediums (DMEN, McCoy, RPMI and water) in the presence or absence of UV light presented alterations, that reflected in some cytotoxicity and genotoxicity tests, once NP-TiO₂ (Anatase e Aerosolox) show different behavior depending on the medium they are dispersed. In Allium cepa tests the nanoparticles presented significant alterations in the concentrations tested (1, 10 e 100 µg/mL). The results reveal that the anatase at 1 and 10 µg/mL presented weaker values significantly different when compared to the control in all treatments. Regarding the differences found between the two types of nanoparticles we could observe that the results presented significant differences for concentrations at 10µg/mL without exposure to ultraviolet light, being the damages on Anatase nanoparticles stronger and at the concentration at 1 and 10µg/mL with exposure to ultraviolet light, being NP-TiO₂ (Anatase e Aerosolox) the studies performed with soil microbiota showed that over time some variation occurred in relation to the type of existing bacteria variation, which has also occurred among the different NP-TiO₂ tested. The physicochemical characterization proved to be essential for the best understanding of the biological effects of NPs. The contribution of the present work for the area of nanoparticles toxicity is of great value as it helps on the understanding of the behavior of these materials, what is essential for their use in a safe way, causing minimum damage to the environment and human beings.

### MO153
**Molecular assessment of bacterial community dynamics and functional endpoints during sediment bioaccumulation tests**

N. Diens, Wageningen University / Department of Aquatic Ecology and Water Quality Management; M.R. Dimitrov, Wageningen University and Research Centre; A.A. Koelnians, Wageningen University / Aquatic Ecology and Water Quality Management Group; H. Smidt, Wageningen University / Laboratory of Microbiology

Whole sediment toxicity tests play an important role in the environmental risk assessment of chemicals. It is not clear, however, to what extent changing microbial community composition and associated functions affect test results. We assessed the development of bacterial communities in artificial sediment during all stages of a 28 day bioaccumulation test with PCBs and chlorpyrifos and four marine benthic invertebrates. Denaturing gradient gel electrophoresis and 454-pyrosequencing of PCR-amplified 16S ribosomal RNA genes were used to characterise bacterial community structure. Abundance of total bacteria and selected genes involved in important microbial metabolic pathways in functional and destructive dioxygenating and competitive PCR. Variables such as sediment organic matter, invertebrate species, and time affected bacterial diversity, community composition, and ecologically relevant functional endpoints, such as abundance of nitrogen-fixing bacteria, ammonia-oxidizing bacteria, denitrifying bacteria, sulphate-reducing bacteria, and bacteria capable of hydrolysing organophosphate compounds. This implies that the bacterial species present, community dynamics is likely to affect chemical degradation, biotransformation, and water quality variables that in turn can affect the outcomes of sediment tests for the target species and consequently the risk assessment. To our knowledge, this is the first work to describe dynamics of bacterial community structure and associated functional attributes during a bioaccumulation test using complementary molecular tools.

### MO154
**Evaluation of native microbial soil populations at a trichloroethylene contaminated Superfund site in the presence of a permeable reactive barrier (biowall) using a metagenomics approach.**
G.T. Nino de Guzman, Department of Civil and Environmental Engineering; C.J. Hapeman, USDA-ARS; L. McConnell, USDA Agricultural Research Service / Environmental Management and Bioprocess Utilization Laboratory; P. Millner, United States Department of Agriculture / ARS; A. Torrens, University of Maryland / Civil and Environmental Eng; B. Kjellerup, Goucher College The Beaverdam Road Landfill occupies 3.5 acres and was an active disposal site for miscellaneous non-hazardous waste from 1943 to 1990, before being capped. In 1994, this site was included on the Nation Priorities List (NPL) for periodic inspection and remediation in accordance with the program regulations. In 2002, trichloroethylene (TCE) was identified in the groundwater as high as 435 ppb, whereas the Maximum Contaminant Level (MCL) in drinking water is 5 ppb. The contaminant source remains unidentified. The construction of a permeable reactive barrier (PRB) was determined to be the least invasive method of remediation. Previous research in this field has focused on rudimentary interactions between PRB materials and TCE either in situ or in lab-scale reactors under ideal conditions, instead of the temperature range common to groundwater (10-15°C), a critical component to TCE behavior. Furthermore, the subsequent microbial colonization of the PRB material from the surrounding soil microbiota has not been sufficiently investigated. The objectives of this study were to determine if any TCE-degrading microbial communities could be found in the site soil and if they could ultimately colonize the PRB material to increase the degradation capability of the engineered structure. DNA was extracted from native soil samples from a highly, a moderately, and an unamended control area at the site to evaluate the microbial communities for TCE degradation potential. DNA was also extracted from the PRB building material and batch short- and long-term laboratory experiments. In the second phase of this investigation, a 16S rRNA sequencing and taxonomic analysis (Illumina HiSeq2500). The presence of tceA, vcrA, and bvcA genes, linked to TCE degradation, were applied for quantitative analysis using qPCR. A commercially available consortium, SDC-9 (Shaw Environmental, Inc., Lawrenceville, NJ), was used as a control to gauge Dronocolodex behavior and presence in this environment. Anaerobic TCE degrading reactors (12°C) were subsequently constructed and packed with mulch, compost, and general-purpose sand either with or without zero-valent iron (ZVI) and/or glycerc to determine TCE degradation potential. If TCE degrading microbial communities were not able to colonize the PRB materials in the field, augmentation options will be explored at the bench scale then evaluated for deployment in the field.

MO155 Microbiome analysis to identify species and biomarkers for water quality monitoring and bioremediation
T. Venkateswarlu, Simon Fraser University / Department of Molecular Biology and Biochemistry; M.A. Peabody, Simon Fraser University / Department of Molecular Biology and Biochemistry; M.I. Uyaguari Diaz, University of British Columbia; M. Pylatuk, Simon Fraser University / Biology; K. Cronin, M.A. Croxen, University of British Columbia; A. Crisan, British Columbia Centre for Disease Control; E.J. Griffiths, R. Lo, Simon Fraser University; H.L. Osachoff, BC Ministry of Environment / Water Protection and Regional Sections; F. Chiaro, V. Hossain, C.A. Suttle, N.A. Prystajecky, J.L. Isaac-Renton, P.K. Tang, University of British Columbia; C. Kennedy, Simon Fraser University; F.S. Brinkman, Simon Fraser University / Molecular Biology and Biochemistry

Using metagenomic sequencing, we are profiling microbial taxa and genes across contaminated watersheds to identify specific genetic biomarkers of pollution associated with a group's behavior and presence in this environment. Anaerobic TCE degrading reactors (12°C) were subsequently constructed and packed with mulch, compost, and general-purpose sand either with or without zero-valent iron (ZVI) and/or glycerc to determine TCE degradation potential. If TCE degrading microbial communities were not able to colonize the PRB materials in the field, augmentation options will be explored at the bench scale then evaluated for deployment in the field.

MO156 Microbial responses to diffuse pollution in seawater
m.villegas@uio.no, M. Fernández-Pinos, IDAEA-CSIC. T. Ailoito, CNAG; B. Pina, IDAEA-CSIC / Environmental Chemistry; X. Irigoyen, King Abdullah University of Science and Technology; J. Dachs, IDAEA-CSIC / Environmental Chemistry

Increasing trace amounts of thousands of man-made organic pollutants (OPs) and hydrocarbons reach seawaters mainly through run-off, riverine and diffuse atmospheric inputs. The fraction of marine dissolved organic matter (DOM) accounted by OPs remains unknown due to the analytical complexity to analyse both pools. However, this assessment is important because OPs are hydrophobic, and thus tend to concentrate in marine biota at higher intracellular concentrations than in seawater. Besides bioconcentration, prokaroyxotes (the main consumers of DOM) can degrade some OPs as indicated by few indirect field evidences. Whereas a handful of studies have shown a significant impact on phytoplanktons, the effects of OP present in seawater (and not whether considering just few OPs), the effects of OPs on heterotrophic microorganisms biodiversity and functionality is unknown. We have applied metatranscriptomic approach in enrichments of seawater from the Atlantic and Pacific oceans adding a complex mixture of naturally occurring organic pollutants at similar concentrations than those found in seawater in order to analyze the effects of the known and unknown fraction of pollutants to the marine picoplankton, including the photosynthetic and heterotrophic fraction. Preliminary results show that the pool of organic pollutants had a higher inhibitory effect on cell performance of phototrophic cells although their abundances were slightly affected, consistent with the non-negative effects of the low concentrations of pollutant mixtures in the treatments. On the other hand, heterotrophic groups of bacteria benefited from the carbon supply of POPs to increase their activities.

MO157 Individual, physiological and molecular stress responses exposed to ionising radiation in Lemna minor
a. van hoekc, N. Hoem, J. Hoem, P. Millner, Simon Fraser University / Biology; K. Cronin, M.A. Croxen, University of British Columbia; C. Kennedy, Simon Fraser University / Molecular Biology and Biochemistry

The biological effects and interactions of different radiation types in plants are still far from understood. Additional knowledge on the impact of ionising radiation in plants on individual, biochemical and molecular level will help to unravel the toxic mode of action. Among different radiation types, gamma radiation treatments have been mostly studied both in lab and field studies to derive the biological impact of radiation toxicity in organisms. However, environmental relevant studies on chronic low-dose gamma exposures are scarce. Exposure to gamma radiation can induce ionization events causing an increase in reactive oxygen species (ROS) and inducing damage to biological material like DNA, lipids and structural proteins. The present study aimed at evaluating individual, physiological and molecular endpoints to understand the mode of action of ionising radiation stress in plants. The floating freshwater plant Lemna minor was chosen as model system. In an effort to strengthen duckweed genomics research, we sequenced the genome of Lemna minor using Illumina sequencing platform. Plants were being exposed to an external gamma radiation source for seven days. The classic growth related endpoints on plant biomass and frond area and frond number, were measured and compared with biochemical and molecular endpoints. A dose-response curve with 60% growth inhibition was obtained on plant growth. Additionally, Lemna plants showed also dose-dependent responses in anti-oxidative defense activities, nuclear Bed biofilm samples from up and downstream of a point source of copper contamination. For

MO158 High-resolution arrays to investigate the occurrence of a stress syndrome in mussel caged in the Baltic Sea
F. Dondero, Universiteit van Piemonte Orientale Avogadro / DSIT; I. Saggese, University of Piemonte Orientale / Department of Sciences and Technological Innovations; T. Turja, Finnish Environment Institute, SYKE / Marine Research Centre; K.K. Lehtonen, Finnish Environment Institute / Marine Research Centre

We used a newly developed high-resolution arrays based on Agilent Technologies Inc. SureDesign to investigate high-throughput transcriptional changes in Mytilus spp tissues (digestive gland) obtained from specimens caged in four previously characterized field sites (S1, S2 in Sundsvall; G1, G2 in Gavle) receiving different point loads and local sources atmospheric inputs. The new microarray encompasses 15444 annotated mussel genes originated from RNA-seq and further sequence data assembly of about 22 million short reads (100 bp paired end sequences) obtained from multi-library of each of G1, G2 and M. trossulus. The new microarray was functionally annotated for biological endpoints to understand the mode of action of ionising radiation stress in plants. As multiple levels in biological organisation of the organism were considered, and also different dose rates taken into account, this approach allows a better understanding of the current state of the art of radiation stress in higher plants. This research was supported by the European Commission Contract Fission-2010-3.5.1-209672 to Strategy for Allied Radioecology (www.star-radioecology.org) and a project of the Fund for Scientific Research (FWO-Vlaanderen, GA040.11N).

MO158 High-resolution arrays to investigate the occurrence of a stress syndrome in mussel caged in the Baltic Sea
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the occurrence of adverse outcome pathways as highlighted from a hyper-genometric classification of GO terms associated to differentially expressed genes. In particular in tissues from specimens sampled at S1 site, pathway analysis showed the implication of genes that may be involved in metabolic disorders linked to aminosugar utilization, endosulfan trafficking, humoral immune response, typical of a pollutant stress-response. Correlation with complementary molecular approach (quantitative PCR) functional data obtained from physiological markers is ongoing to identify robust predictors of stress syndrome occurring in a low salinity environment such as the Baltic Sea.

MO159 Application of RNA-seq in Brassica oleracea toward molecular ecotoxicological studies
S. Dumez, Université Lille 2 / Laboratory of plant and fungal sciences; F. Brulle, Unité Expertise in Toxicology Ecotoxicology of Substances DRC VIVA ETES; D. Cuny, Université de Lille 2
Brassica oleracea (cabbage) is one of the selected species recommended by OECD for the testing of chemicals in soils but this plant has also already been employed for atmospheric pollution biomonitoring purposes. Despite its close relationship with the model plant Arabidopsis thaliana, and saw its genome partially been sequenced, few sequences are available in the Genbank nucleotidic database and a precise functional annotation is still missing. Nowadays, New Generation Sequencing methods (NGS) represent useful technologies that respond quickly to a lack of molecular data in non-model species and allow a fast obtaining of a huge amount of information needed to conduct functional and toxicological research. These techniques allow the sequencing of all or a part of a genome or a transcriptome, the latter in order to get extensive cDNA information. Here we introduce the transcriptome sequencing of Brassica oleracea var. viridis leaves by using 454 pyrosequencing. Normalized cDNAs from 9 different experimental conditions yielded 275 446 expressed sequence tags (ESTs), assembled in 33 925 contigs and 42 654 singlets. By using Blast2go online software, we found that most ESTs (E-value ≤10–8) and, based on Gene Ontology classification, functional annotations were recovered for 76.3% of them. 43% of blasted sequences found homologies with those of the model-plant A. thaliana. KEGG seqs show that the annotated sequences are associated with 140 metabolic pathways including xenobiotics biodegradation and metabolism, signaling molecules, etc… up to 0.8% of the transcripts produced, we found numbered stress responsive genes and cell defense related genes (enzymatic anti-oxidative machinery, DNA repair, cytochromes P450 coding genes…), all being potentially of interest for monitoring the effects of pollutants at the molecular level in the leaf of B. oleracea. All of these results make ecotoxicogenomic studies now possible in this plant species.

MO160 Comparison of different DNA extraction methods for marine peripatery and its implications for ecotoxico-metagenomics
N. Corcorl, Department of Biological and Environmental Science; M.K. Eriksson, Chalmers University of Technology / Department of Shipping and Marine Technology; T. Tóth, University of Gothenburg / Department of Biological and Environmental Sciences
This is a time with unprecedented capacity of DNA sequencing technologies for environmental monitoring. These techniques allow the sequencing of all or a part of a genome or a transcriptome, the latter in order to get extensive cDNA information. Here we introduce the transcriptome sequencing of Brassica oleracea var. viridis leaves by using 454 pyrosequencing. Normalized cDNAs from 9 different experimental conditions yielded 275 446 expressed sequence tags (ESTs), assembled in 33 925 contigs and 42 654 singlets. By using Blast2go online software, we found that most ESTs (E-value ≤10–8) and, based on Gene Ontology classification, functional annotations were recovered for 76.3% of them. 43% of blasted sequences found homologies with those of the model-plant A. thaliana. KEGG seqs show that the annotated sequences are associated with 140 metabolic pathways including xenobiotics biodegradation and metabolism, signaling molecules, etc… up to 0.8% of the transcripts produced, we found numbered stress responsive genes and cell defense related genes (enzymatic anti-oxidative machinery, DNA repair, cytochromes P450 coding genes…), all being potentially of interest for monitoring the effects of pollutants at the molecular level in the leaf of B. oleracea. All of these results make ecotoxicogenomic studies now possible in this plant species.

MO161 Impact of Cd and Pb impregnation on the health of an adult population neighbouring a landfill : a case study
M. Cabral, Université Cheikh Anta Diop; A. Verdín, Université du Littoral Côte d’Opale / Unité de Chimie Environnementale et Interactions sur le Vivant UCEIV EA; G. Garcon, Université de Lille 2; A. Touré, C. Diop, M. Fall, Université Cheikh Anta Diop; S. Boushsina, D. Dewaeye, F. Cazier, Université du Littoral Côte d’Opale; A. Tall Diao, Université Cheikh Anta Diop; P. Shiral, Université du Littoral Côte d’Opale; A. Douf, Université Cheikh Anta Diop
This case-control study dealt with the health adverse effects within the population neighbouring the Mbeubeuss waste dump, which is located near the district of Malika (Diamalaye II) in Dakar (Senegal). All the household and industrial waste arising from Dakar are stored in this open landfill without being covered and are therefore potential sources of Pb and Cd contamination of environmental samples. To test these hypotheses, the objective of this study is part of improving the health of the population neighbouring Mbeubeuss by determining Pb and Cd concentrations both in environment and humans, and studying possible renal function alterations within the adults. Soil and air samples were collected in the control site (Dorado Salam) and the waste dump neighbouring site (Diamalaye II). Control and exposed adults were recruited (N=52) and in Dakar city, 28 adults were recruited (N=28). Pb and Cd concentrations in soil, air and biological samples were determined. Moreover, we were interested in analyzing some impegmination (zinc protoporphyrin, δ-aminolevulinic acid dehydratase) and oxidative stress biomarkers (malondialdehyde, glutathione status), in addition to several nephrotoxicity parameters (creatininuria, proteinuria, lactate dehydrogenase, CC16 protein, glutathione S-transferase-alpha and retinol binding protein) in blood and/or urine. The results showed the significant Pb and Cd contamination of the soil and air samples derived from the landfill, and therefore of the neighboring population of adults. This critical exposure to environmental Pb and Cd had some harmful consequences for their health, as shown by the reported oxidative stress and nephrotoxicity signs.

MO163 -Omic profiles of Xenopus A6 kidney cells by ATR-FTIR or Raman spectroscopy and LC-MS with computational analysis
E. Gorchuchegui, Analytical Environmental Chemistry; C. Porte, CSIC - IQAB / Environmental Chemistry; S. Lacorte, IDAEA-CSIC / Environmental Chemistry; R. Tauler, IDAEA-CSIC. F. Martin, Lancaster University
Keywords: Omic LC-MS/I/IR/Raman profiles, Xenopus laevis (A6), Chemometrics The exposure of organisms to environmental pollutants, such as perfluorooalkylated substances (PFASs), can result in unknown effects in their mRNA, protein or metabolic system. Thus, the study of their –omic profiles appears as an urgent issue since it can provide very meaningful information about the biological responses of the organisms exposed to potentially toxic environmental insults. Infrared or Raman spectroscopy techniques have been developed as excellent tools for initial characterisation, providing information of the molecular
composition of the interrogated biological specimens. Moreover, high resolution mass spectrometric techniques appear to be powerful tools for further characterisation, providing information on elemental composition and thus, allowing the identification of potential biomarkers. However, the large amounts of data generated with such techniques complicates the extraction of distinguishing features. **Chromometric methods**, such as principal component analysis (PCA), have evolved as powerful tools to address this issue, enabling fast data processing. Moreover, multivariate curve resolution-alternating least squares (MCR-ALS)\(^2\) allows for a distinct resolution of the profiling problem in LC-MS, which results in a better identification of unknowns\(^4\). In the present study, the effects of **four PFASs**, namely perfluorobutanesulfonate (PFBS), perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) were studied in the model bacteria *Escherichia coli* (K12) kidney cell line by attenuated total reflection Fourier-transform infrared (ATR-FITR) spectroscopy, Raman spectroscopy and ultra-high performance liquid chromatography coupled to time-of-flight mass spectrometry (UHPLC-TOF-MS). Particular emphasis was placed on the analysis of their effects on cellular lipids in an attempt to evaluate the individual effects of the four PFASs on cellular membranes, which were previously determined for a mixture of eight PFASs.\(^5\) Results revealed both a lipid and membrane disruption produced by the xenobiotics, which occurred differentially in cells with and without domes;\(^6\) cell layer evolutions produced by a water inflow between basal membranes and culture flask. Finally, electron microscope images allowed the visualization of the structural changes produced in cellular membranes caused by the presence of PFASs.

**MO164** Development of an embryotoxicity test for *Enchytraeus crypticus* - unravelling the mechanisms of Cd

M. Gonçalves, University of Aveiro, Department of Biology & CESAM / Biology; R. Queiroz, Universidade de Aveiro / CESAM Department of Biology: A. Réma, ICBAS University of Porto; A. M. Soares, Universidade de Aveiro / Department of Biology and CESAM; A. Faustino, Universidade do Porto; M. Amorim, Universidade de Aveiro / Department of Biology and CESAM

The existing standard enchytraeid reproduction test (ERT) concerns the assessment of effects on survival and reproduction. In the present study we optimized and proposed an embryotoxicity test using *Enchytraeus crypticus*. Cadmium (Cd) was used as a test substance. Endpoints evaluated were embryo development, number of embryonic structures, Calcium (Ca) channels quantification and hatching success with macroscopic monitoring, histological and immunohistochemistry analysis. Results showed that Cd is embryotoxic for this species, causing a decrease in the hatching success, a delay or disruption in formation of embryonic structures depending on concentrations.

**MO165** Performance evaluation of dimension reduction methods in improving the clustering of toxicants based on high dimensional toxicogenomic data

S.M. Rahman, Northeastern University / Civil Environmental Engineering; C. Gao, Northeastern University / Bioengineering; A. Gu, Department of Civil & Env Engineering / Department of Civil Env Engineering / Biotechnology Initiative Program

The information-rich toxicogenomic data not only enables potential toxicity mechanisms identification and prediction, but also allow chemical clustering based on their toxicogenomic profiles. Due to presence of many genes and their temporal patterns, the high dimensional gene expression data present a high degree of complexity in terms of redundant and noisy information, complexity and availability of computation algorithms and computation time. Dimension reduction techniques project the high dimensional data into the lower dimensional subspace and can be applied to overcome the curse of dimensionality. The current study performed comparative evaluation of the clustering performances using two dimension reduction techniques, Principal Component Analysis (PCA) and Multi-dimensional Scaling (MDS). In addition, how the distance metric such as Euclidean and Correlation metric adopted during clustering analysis may impact their overall performances is evaluated. The stress response ensemble whole-cell 100 GEP-Fused Ecoli / K12, MG1655 reporter strains was employed, which covers all known genes involved in stress responses. For every altered gene expression profiles for 24 selected chemicals at 6 different dose concentrations were monitored over a period of 2 hours at 5 minutes intervals. Consensus clustering was applied to the original dataset with all 2450 dimensions which comprises with altered expression profiles of 98 genes over 25 time points, as well as the lower dimensional datasets derived from the application of both PCA and MDS. Clustering analysis of the lower dimensional datasets per clusters with higher separations resulting higher no. of groups. PCA and MDS generated lower dimensional datasets consumed less than one-third of the time required to cluster the original higher dimensional dataset. Correlation distance based clustering led to low resolution separation with consensus index values but did not separate responses of the some chemicals based on its concentrations, as for each chemical all the 6 doses are grouped in the same partition. While, Euclidean distance based algorithm separates the lower and higher doses of same chemical in separate groups resulting higher no. of clusters. Finally clusters generated from different datasets were compared with the clusters of original dataset by estimating Rand index and Adjusted Rand index, which find the probability of agreement among the clustering results.

**MO166** Identification of genes and enzymes involved in mesotrione biodegradation by *Bacillus megaterium* I.CARLES; P. Besse-Hoggen, C. Bardot, I. Batissen, Université Blaise Pascal Mesotrione is a selective herbicide belonging to the triketone family commonly used in corn or the like since 2003. In order to predict its potential toxicological impacts, the environmental fate of this herbicide and the molecular mechanisms underlying stress responses in non-target microorganisms have to be identified. These points are not well documented so far. A mesotrione-degrading strain, *Bacillus megaterium* IB11, isolated from an agricultural soil was used as a reference model to study the biodegradation dynamics.\(^1\) Three metabolic pathways were identified coming from the reduction of the nitro group of mesotrione leading to hydroxylamine derivatives, and then from hydrolysis reactions leading to the final accumulation of AMBA (2-amino-4-methylsulfonylbenzoic acid) in the medium. The aim of this research work was to study the metabolic shifts observed in strain IB11 after exposure to mesotrione and to identify the enzymes involved in its degradation. The metabolic response of the strain IB11 to mesotrione stress was studied by comparing the intracellular proteomes of mesotrione-treated and control cultures by a two Dimensional-Differential In Gel Electrophoresis approach (2D-DIGE). Sixteen proteins mainly involved in stress, metabolic and storage mechanisms were identified. However, the network of these differential proteins allowed us to emphasize a direct link with nitroreductase (NR) enzymes. These results are consistent with the chemical structures of metabolites produced during mesotrione degradation and suggest the implication of a NR in the transformation of mesotrione into AMBA. To test this hypothesis, 9 genes coding for nitroreductases, showing 11% to 64% of similarity in their amino acid sequences, were amplified in IB11 strain and the corresponding proteins produced in expression vector. Four of them showed an up-regulation and one showed 20% transformation of mesotrione. Activity tests of the other NR towards mesotrione are under progress. This study constitutes the first identification of genes/enzymes involved in mesotrione biodegradation, allowing a better knowledge of mesotrione biodegradation pathways. These genes and/or enzymes may also be used as biomarkers to predict the capacity of ecosystem to degrade mesotrione and to assess the potential risk of this compound on the environment through the food web.\(^2\)

**MO167** Prioritization and management of multiple stressors in river basins: effects of chemical contaminants and natural stressors (P)

M.F. Sanchez, Groundwater Management; H. Duel, A. Alejos Sampedro, IB11, Universitat Rovira i Virgili / Geosciences; I.Eglesias / Forest Ecology and Management; S. Perez-Luzardo, Universitat Rovira i Virgili / Environmental Chemistry; F. Gao, Northeastern University / Bioengineering; A. Gu, Department of Civil & Env Engineering / Department of Civil Env Engineering / Biotechnology Initiative Program

Prioritization and management of multiple stressors in river basins: effects of chemical contaminants and natural stressors (P)

In the project MARs (Managing Aquatic ecosystems and Water Resources under multiple stress), experiments and predictive models at local, basin, and European scale will be used to assess the combined impacts of multiple stressors affecting water quantity and quality, ecological status, ecological functions, and ecosystem services under contrasting scenarios. Various future climatic and socio-economic scenarios were chosen to define three storylines at the European level, based on the latest versions of the Shared Socioeconomic Pathways and the Representative Concentration Pathways. The combinations SSP5 and RCP8.5, SSP3 and RCP8.5 and SSP2 and RCP4.5 were selected through a participatory process as starting points. These storylines differ mainly in four main aspects; main drivers in the economy, economic, growth policies regarding the environment, and public concern about the environment and protection of ecosystem services. The storylines had been downscaled to the three basin regions defined within MARs using the expert knowledge of the scientists working in the basins, and the stakeholders within those basin regions. In order to simulate the future scenarios at both basin and European scale, and to assess the impacts of multiple stressors, quantitative values for the impacts of ecosystem service degradation for each region were required. Several projects and modelling tools were reviewed with the aim of identifying the data required to support the selected storylines. The data was derived mainly from the previous projects and tools, including CLIMSAVE, ISIMP, BASE, SCENES, and IMAGE. Values for diverse climate variables, runoff, water abstraction, potential flood plains, nutrient diffuse source emission, land use, population and GDP were collected. The result is a suite of quantitative values for diverse parameters and variables in grid cell or vector format, which range from daily to yearly time steps at resolutions ranging...
MO168 Evaluation of ecosystem services of aquatic ecosystems under water scarcity - a methodological framework to elicit stakeholders’ perspective

L. Mustajärvi, Altterra

Freshwater ecosystems are under threat from the effects of multiple stressors, including organic and inorganic pollution, land use changes, water abstraction, invasive species and pathogens [1]. Little is known beyond the described effects of single stressors on the chemical and ecological status of water bodies and on their ecosystem functionality. This lack of knowledge limits our capacity to understand ecosystem responses to multiple stressors and to define a programme of measures that can improve the ecological status of a water body as sought by the European Water Framework Directive. Decision support system tools supporting the implementation of the WFD including land use scenarios are commonly based on the DPSIR model. The Economics of Ecosystems and Biodiversity (TEEB) approach provides a framework for assessing multiple stressor. Economic valuation includes monetary and non-monetary methods. Monetary methods although under continuous development and construction are well documented. Non-monetary valuation relies on perception and values of stakeholder at large. The objectives of this article are to i) adapt the TEEB framework for the assessment of aquatic ecosystems under multiple stressors, ii) to elicit Economic valuation of ecosystem services from the viewpoints of stakeholders at European level and to identify trade offs as well as multiple benefits and iii), to provide a conceptual bridge between the DPSIR model and TEEB approach to enrich programme of measure to aim toward Good ecological status. The diagramme below presents the methodological framework to bridge TEEB framework with the DPSIR model along 3 fields work activities in 6 river basin affected by water scarcity in Europe (Italy), Sahel/SouthwestAsia, Ebro (Spain), Greec, Alemannian (England) and Sousa Masas (Morocco). The field works includes two participative workshop with the water framework directive community (stakeholders) in each river basin. The first participative workshop aims to elicit their perspectives, understandings and values, and to comment on scenarios and to test the plausibility of computer based model output. Follows the questionnaire survey that hold to prioritise ecosystem services benefits and to test potential programme of measures amongst the river basin population. The final workshop aims to validate previous finding, to present output from land use model and to test acceptance for proposed measures with initial stakeholders.

MO169 Development of an Integrated Methodology for the Sustainable Environmental and Socio-economic Management of the Water Resources Ecosystem.

I. Souliotis, P. Koundouri, ATHENA

The development of the Water Framework Directive aimed to establish an integrated assessment methodology at European level. This framework revolves around inland surface waters, transitional, coastal waters and ground waters. In the process of achieving the environment and ecological objectives set from the Directive, the role of economics is put in the core of the water management. An important feature of the Directive is the recovery of total economic cost of water services by all users. The total cost of water services can be distributed into individual financial and resource costs. Another important aspect of the directive is the identification of major drivers and pressures in each River Basin District. The presentation describes a methodology that can be followed in order to achieve sustainable and environmental and socioeconomic management of freshwater ecosystem services. The ecosystem services approach is in the core of the suggested methodology for the implementation of a more sustainable and efficient water management. In a nutshell, this approach consists of the following three steps: (i) socio-economic characterization of the River Basin area, (ii) assessment of the current recovery of water use cost, and (iii) identification and suggestion of appropriate programs of measures for sustainable water management over space and time. This methodology is consistent with a) the economic principles adopted explicitly by the Water Framework Directive (WFD), b) the three-step WFD implementation approach adopted in the WATECO document, c) the Ecosystem Service Approach to valuing freshwater goods and services to humans. The presentation starts with the economic aspect and implementation of WFD, continues with the description of Ecosystem Services Approach and ends with the description of the steps and sub-steps of the three-step methodology. The presentation analyses how the effects of multiple stressors and socio-economic development can be quantified in the context of freshwater resources management.

MO171 Determining the release of hydrophobic organic contaminants from sediment by in-situ benthic flow-through chambers

L. Mustajärvi, Altterra

This study presents a novel in-situ benthic flow-through chamber for determination of release of hydrophobic organic contaminants (HOCs) from sediment to water. Sobek et al. (2014) observed high chemical activity ratios between pore water and bottom water for dioxins in coastal areas of the Baltic Sea, demonstrating a potential release of dioxins from sediment to the water column. Lab and modelling studies (Thibodeaux et al. 2003 and Granberg et al. 2008) have shown that bioturbation is an important parameter increasing the release of HOCs from sediment to water. In-situ benthic flow-through chambers measure the release of HOCs from sediment related to bioturbation have not been done previously. The design of our novel benthic flow-through chamber was developed based on a closed benthic chamber, which has proven useful in assessing diffusive flux of HOCs from sediment (Eek et al. 2010). Since the previous version of the chamber was closed to ambient water, oxygen deficiency in the chamber could stress the benthic fauna and lead to starvation of bioturbation activity. This means that the closed chamber does not measure the HO flux as it occurs under natural conditions. The design of the flow-through benthic chamber enabled measurement of sediment to water flux while the water in the chamber was kept at ambient oxygen concentration by constantly pumping ambient water through the chamber. In order to measure flux from the sediment enclosed by the chamber, incoming water was stripped of its content of HOCs by pumping the water through a sorbent. HOCs released from the sediment was captured on a second sorbent in the water flow from the chamber. The relative importance of bioturbation can be studied by comparing flux measured with a flow-through chamber and flux measured by a closed chamber. The advantages of the in-situ determination of flux over flux determined in lab or by modeling are: 1) it yields a direct measurements of the flux with the last sample collected at the last day of the experiment and 2) it does not require the assumption that the flux rate is constant during the exposure.

MO172 Preliminary biodegradation studies of different commercial homo-polymers by MALDI-TOF MS technique for the evaluation of river metabolism

D. Rivas, IDA-CIES/CSIC; D. Barcelo, IDA-CIES/CSIC; P. Koundouri, ATHENA

The field works includes two participative workshop with the water framework directive community (stakeholders) in each river basin. The first participative workshop aims to elicit their perspectives, understandings and values, and to comment on scenarios and to test the plausibility of computer based model output. Follows the questionnaire survey that hold to prioritise ecosystem services benefits and to test potential programme of measures amongst the river basin population. The final workshop aims to validate previous finding, to present output from land use model and to test acceptance for proposed measures with initial stakeholders.
Metabolomics and Pollution Sourcing: Molecular Markers in the Field (P)

MO174 Non-targeted HPLC-HRMS metabolomics for studying the effects of cadmium and copper exposure in Japanese rice

M. Navarro, IDAEA-CSIC / Environmental Chemistry; J. Jaumot, IDAEA CSIC / Environmental Chemistry; R. Tauler, IDAEA-CSIC

During the last centuries, the level of heavy metals on soil and surface water has increased significantly. These pollutants can be found as constituents of the Earth’s crust and geological processes, but human activities, such as mining, agriculture and a wide range of industrial activities, can drastically alter heavy metal geochemical cycles. Cadmium and copper pollutants can be bioaccumulated through the food chain. Since the diet is the main source of ecotoxicological exposure for the general population, intensive research has been performed on the accumulation of these pollutants in edible plants, such as in rice (Oryza sativa japonica). For this purpose, rice was cultivated at optimal conditions during 10 days and after this period, it was exposed to different concentrations of cadmium and copper (from 10 to 1000 µM) for 10 days more. After harvesting, rice leaves were employed for analysis. A non-targeted HPLC-HRMS method has been employed coupling an HILIC TSK gel Amide-80 column (Tosoh Bioscience 5µm, 250 mm, 2.0 mm i.d.) with an LTQ-Orbitrap mass spectrometer (Thermo Scientific). Different multivariate data analysis tools (Principal Component Analysis, Partial Least Squares - Discriminant Analysis and Multivariate Curve Resolution) have been used to discriminate between control and treated samples and to identify which molecular markers were the most relevant for this classification. Finally, KEGG database was used to evaluate the metabolic pathways changes underlying cadmium and copper exposure. The research leading to these results has received funding from the European Research Council under the European Union’s Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement n. 32073 [1] A. D’Alessandro, M. Taamalli, F. Gevi, A.M. Timperio, L. Zolla, T. Ghnaya, T. Nebot, 12 (2013) 4979-4997. [2] N. A. Alsham, T. Nakamura, S. Komatsu, Amino Acids 42 (2012) 317-327. [3] F. Villiers, C. Ducruix, V. Hugouvieux, N. Jarno, E. Ezn, J. Garin, C. Junot, J. Bourguignon, Proteomics 11 (2011) 1650-1663.

MO175 Towards standardized validation and reporting of non-targeted metabolomics approaches for routine analysis and regulation

J. Riedl, Safety in the Food Chain; S. Esslinger, C. Faulh-Hassek, BFR Federal Institute for Risk Assessment / Safety in the Food Chain

Metabolomics approaches have been demonstrated to be successful in many research studies of various fields, but their application in routine analysis or implementation in regulatory frameworks is still in its infancy. Regarding the huge amount of data that is generated by non-targeted metabolomics approaches, challenges that have to be faced are i) to distinguish study-relevant information from unintended systematic variation and noise and ii) to guarantee the robustness of results and their reproducibility. Several development protocols do exist, for non-targeted metabolomics, typically based on spectroscopic or spectrometric measurements and a subsequent multivariate statistical analysis, such strategies have not yet been developed. A review of literature with regard to validation strategies, in this case on food authentication using metabolic fingerprinting techniques, shows that the development of an industry specific validation application of metabolomics approaches: a lack of validation strategies for the metabolomics workflow and limited reporting on data quality. In particular, model validation within the experiment was well addressed, but the accuracy of the results considering experiment-to-experiment, instrumental, operator or laboratory variation was hardly considered. Moreover, data processing was little transparent in many studies with hardly characterised metabolic fingerprints used for data evaluation. On this basis, recommendations and future work for validation and reporting of non-targeted metabolomics are derived to progress standardization for a potential implementation of such approaches in routine analysis for food control purposes. These suggestions of “good practice” for validation and reporting might also be relevant for other applications within regulatory frameworks.

MO176 UNTARGETED METABOLOMIC ANALYSIS FOR THE ASSESSMENT OF BIPHENOL-A EFFECTS ON ZEBRAFISH EMBRYOS

E. Ortiz, IDAEA-CSIC / rotamericidesecies; J. Jaumot, IDAEA CSIC / Environmental Chemistry; B. Pina, IDAEA-CSIC / Environmental Chemistry; F. Benvenete, V. Sainz-Nebot, University of Barcelona; R. Tauler, IDAEA-CSIC / Zebrasigo (Danio rerio) is a model vertebrate organism for biological, behavioural and biomedical research. In particular, zebrafish embryos are a well-recognized model for environmental risk assessment of chemicals due to their rapid development into larvae (within 48 hours) and to their high sensitivity to chemical treatments. In addition, their small size, wide distribution and ease of conditions offer the possibility to perform small-scale and high-throughput analyses for -omics studies, including metabolic profiling [1]. Biphphenol A (BPA) is a chemical produced at large-scale for use in the production of plastics and epoxy resins. Furthermore, BPA is an endocrine disruptor that interferes in the endocrine system of aquatic biota at concentrations often found on certain ecosystems. In this work, the effects of biphphenol A were tested on a low concentration of 4 µg/ml (which is LOEC (Lowest observed effect concentration)) on metabolic profiles of five-day-old zebrafish embryos had been evaluated. For this purpose, an untargeted metabolomic approach coupling chromatographic separation by hydrophobic interaction liquid chromatography (HILIC) with mass spectrometric detection and advanced chemometric tools was used to evaluate the metabolic changes induced by BPA exposure in the zebrafish embryos. HILIC-HPLC-MS data were analyzed by a combination of different chemometric tools such as Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA) and Multivariate Curve Resolution (MCR) [2]. The results show a clear discrimination between control and stressed samples and also allow the identification of several metabolites potentially related to the BPA exposure on the basis of their characteristic mass spectra. Finally, this list of identified metabolites allows obtaining information on the metabolic pathways and mechanisms affected by the BPA exposure. This research has been funded by the European Research Council under the European Union’s Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement n. 32073. [1] H. Xu, M. Yang, W. Qiu, C. Pan, M. Wu, Environ. Toxicol. Chem., 32, 1793-1799 (2013). [2] R. Tauler, J. Chemometrics, 24, 87-90 (2010).

MO177 Metabolomics responses to PAHs in the mussels Mytilus galloprovincialis under different nutritive conditions

M. Almoguera, Instituto Español de Oceanografía / Centro Oceánográfico de Dézima; A. Sevilla, University of Murcia; J.A. Campillo, Instituto Español de Oceanografía; C. González-Fernández, Spanish Institute of Oceanography / Marine ecosystems; L. Vinas, Spanish Institute of Oceanography; A. Franco, Instituto Español de Oceanografía; C. Bernal, University of Murcia; A. Lozano, University of Murcia; M. Canovas, University of Murcia; J. Bellas, Centro Oceánico de Vigo / CSIC (Spain)

The results of biological effects obtained in biomonitoring studies, mainly those covering a wide geographical area, are sometimes affected by the large amount of natural variables affecting the biological processes considered as indicators of pollution (biomarkers). One of major source of this natural variability is the particular trophic condition for each sampling site which can also vary between sampling times. Previous studies published by our group in large-scale programs highlighted the influence of nutritional mussel condition on traditional biomarkers measurements. Environmental metabolomics represents an emerging approach to assess the health status of organisms and the impact of marine pollution on them. This –omic aims to study a set of metabolites/low molecular weight intermediates,
which vary according to the physiology, development or pathological state of the target organ. Metabolomics is being considered a powerful tool to characterize the metabolic responses of an organism to both natural and anthropogenic variables that can occur in its environment. The main objective of this study was to determine the relative importance of two variables: food availability and the presence of toxicants, on the metabolic profile of digestive gland of the mussel, Mytilus galloprovincialis, in order to distinguish changes in the metabolism induced due to food scarcity and toxicity and/or the food condition. The metabolic and trophic conditions were simulated by regulating daily food ration of mussels for 45 days: low food condition (N1), medium food condition (N2) and high food condition (N3) which promoted negative, and two positive mussel energy balances, respectively. In all cases, mussels were exposed to two nominal fluoranthene (FLU) concentrations (3 and 0.1 μg L−1), for 3 weeks. On the whole, mussel nutritional condition showed a larger effect than pollution exposure, on the metabolic profile. However, there were some specific metabolites that responded both to animal condition and to the presence of the toxicant.

MO178 Metabolite profiling of 3 benthic invertebrates exposed to wastewater treatment plant effluents by nanoLC-HRMS: a stepping stone to a better understanding of the inter-species response diversity
A. Berlioz-Barbier, I.S.A.; A. Bulette, Institut des Sciences Analytiques UMR TRACES Team / Service Central d’Analyse; J. Garric, Istrea Lyon; E. Vulliet, CNRS
Within the Water Framework Directive (WFD), there are now regulatory arguments in favor of using biota for assessing contamination trends in water body. However, for risk assessment purposes, biological effects on organisms depend on the species of sentinel organisms, the concentration of active toxicant that reaches the target site, and the exposure conditions. In this context, the understanding of the inter-species response diversity needs to be improved to assess the relevance of invertebrates (especially benthic invertebrates) as bioindicators. Metabolite profiling is critical in many aspects of the life sciences and can be used in multiple ways to assess human impacts in the environment. However, obtaining precise information on the chemical composition of complex natural extract (metabolomes) that are primarily obtained from microorganisms is a challenging task that requires sophisticated analytical methods. In this respect, significant advances in hyphenated chromatographic technique such as LC-HRMS, as well as data mining and processing methods, have occurred over the last decade. Moreover, the developments of miniaturized LC strategies such as nanoLC have been studied to improve the sensitivity and increase the number of detected compounds, especially endogenous compounds that are present in small amount. The general aim of this study was to develop a metabolite fingerprinting by using an innovative approaches based on injection of sub-microliter volumes of benthic invertebrate extracts directly on to a nanoLC-nanoESI-HRMS platform. This high-throughput analysis allowed a rapid classification of different samples. The samples consisted of several organism extracts belonging to three benthic invertebrate species. Test organisms were exposed to wastewater treatment plant effluents via caging procedures and laboratory experiments. The purpose of this method was not to identify each individual metabolite, but to compare patterns of metabolite that change in given biological systems. Multivariate data analyses have been applied in fingerprinting to determine differences and classify samples.

MO179 Chemical Pollution Source Markers for Animal and Pharmaceutical Wastes
J.H. Harwood, J. Hendricks, S.L. Narayanay, Tennessee Technological University / Chemistry
We are developing and testing LC-MS analytical methods to identify the sources of organic pollution in streams and rivers. The methods use “molecular markers”, compounds which indicate specific sources of pollution. The artificial sweetener sucralose is an excellent candidate marker of human wastewater input. The compound is stable, resisting both metabolism and degradation in wastewater treatment, and is present in analyzable concentration in waters. We are investigating correlation of this marker with the presence of estrogenic compounds and carbamazepine in domestic wastewater. In our initial study we sampled inflow and effluent from a wastewater treatment plant (WWTP). The WWTP was chosen, because it is a large WWTP with a wide range of influent sources, and because it is a model plant for testing new analytical methods. The samples were analyzed by LC-MS/MS with the electrospray ionization source (ESI). Both grab and POCIS samples were obtained and analyzed for the estrogen estrone 17β-estradiol and 17α-ethinylestradiol. The profiles of fecal sterols associated with particulates have been used to identify waste inputs from specific animals. We are investigating adding bile acids to this sterol profile for more detailed analysis of the animal litter. The steroids we have chosen, cholesterol, stigmasterol, stigmastanol and coprostanol, are characteristic of human and agricultural animal waste. These steroids do not ionize on the LC-MS ESI source, and we have developed the common alternative atmospheric pressure ionization (APCI) for this application. The characteristic bile acids we analyze are cholic acid, chenodeoxycholic acid, deoxycholic acid, hyodeoxycholic acid and lithocholic acid. We have developed methodology to separate and analyze the bile acids and fecal sterols commonly suggested as molecular markers to distinguish pollution from different animal species can be separated and analyzed in a single LC-MS analysis. Results for analysis of municipal wastewater and a local stream impacted by cattle production will be presented.

MO180 Assessment of artificial sweeteners as molecular markers to detect sewage contamination in Asian and African waters in comparison to traditional and emerging markers
H. Takada, Tokyo University of Agriculture and Technology / Laboratory of Organic Geochemistry; S. LOQ, US EPA; T. Ichihara, I. T. Hokajärvi; J.W. Santo Domingo, US Environmental Protection Agency / Cincinnati; T. Huttula, Finnish Environment Institute / Freshwater Centre
We surveyed Vietnam, Thailand, Philippines, Indonesia, Japan, Mozambique, South Africa, Kenya, and Ghana by visiting 358 locations in 2010 – 2014. The water samples including sewage, urban and rural river water, and groundwater were analyzed for artificial sweeteners (Acesulfame : ACE; saccharin : SAC; cyclamate: CYC). Antibiotics were also measured. Potential sewage inputs were identified by alkylbenzenes (LABs) and coprostanols were determined. LC-MS/MS coupled with online solid-phase extraction enabled sensitive analysis of ACE with 2 ng/L of detection limit and 5 ng/L of LOQ. Among 4 artificial sweeteners, ACE was most frequently detected (228 samples among 358 samples) due to its wide usage and low detection limit. ACE concentrations in sewage and heavily-sewage impacted water were ~ 50,000 ng/L in the countries studied, except for Ghana, Kenya, and Thailand with ~ 5,000 ng/L. SAC was less frequently detected (59/358) due to their higher detection limit (100 ng/L) and less usage in some low-income countries probably because of their higher price. Because CYC and SAC are susceptible to biodegradation, they were also less frequently detected in surface waters. However, their higher abundance (~, higher CYC/CYACE ratio) together with lower IE ratio were used to distinguish sewage water. Interestingly, microbial communities from rural rivers in Ghana, no ACE was detected whereas veterinary antibiotics (i.e., tetracyclines) were detected, suggesting the inputs of animal waste. In groundwater in Japan, ACE was widely detected in shallow (~ 10 m) aquifer whereas CYCA was detected in deeper (~ 100 m) aquifer because later was used in the past (~ 1960s). However, artificial sweeteners were rarely detected in groundwater in African countries. In conclusion, ACE has proved to be the most sensitive and reliable marker to assess sewage inputs to surface waters in Asian and African countries. Combination of multiple markers increased accuracy and reliability of the detection and provided further information on types of sewage.

MO181 Sewage effluent discharges as a driver of activity and pathogenicity change in the microbiome of Kokemäenjoki River watershed
T. Pitkänen, National Institute for Health and Welfare / Water and Health Unit; B. Jayaprabash, National Institute for Health and Welfare / Environmental Microbiology Unit; J.K. Juntunen, Finnish Environment Institute / Freshwater Centre; A. Kauppinnen, National Institute for Health and Welfare / Water and Health Unit
The transmission pathways of waterborne pathogens and their markers were studied in the Kokemäenjoki River water river course in Finland. The water flows through hypersaline plants and the river waters are used for producing artificial ground water. Besides energy and water supply, the whole river reach is providing also other important ecosystem services like beaches (e.g. three large EU-level bathing sites), water sports and summer cottage sites. Their values are affected by human-induced contamination as the purified water is used to the Kokemäenjoki River. In this study, the fate of faecal pollution was followed from treated sewage effluent discharges to the raw waters used for drinking water production. We used molecular source tracking markers and consumer chemicals to determine the extent of human-induced contamination in several surface water locations along the Kokemäenjoki River. Pathogen specific viral and bacterial analyses as well as deep sequencing (NGS) of 16S rRNA transcripts (rRNA) and 16 S rRNA genes (rDNA) of microbial communities were conducted. The hydrological transport model of the river was used to evaluate the transmission pathways of the treated sewage at the selected reaches of the river. The associations between the numbers of human-specific microbial and chemical markers (i.e. HF183, PPMV and acetylsalicile-K), microbiome activity levels (i.e. rRNA/rDNA ratios) and the presence of pathogenic microbes (i.e. Campylobacter, Salmonella, Cronobacter) in the river water were analyzed. The results were evaluated by applying ruminant-, swine- and gull-specific molecular markers targeted to rRNA and rDNA. The characterization of the environmental water microbiome structure using 16S rRNA gene and transcript targeted next generation sequencing approach showed that factors other than human impacts may affect most of the active members of the water microbiome. However, the pathogen- and host-specific analyses were crucial in identifying the link between wastewater discharges and the microbial activity and pathogenicity detected in the Kokemäenjoki River water
course. The results of this study provide a unique insight on the fate and transport of faecal bacteria in this watershed.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (P)

MO182
Trace metals (Zn and Hg) and Metallothionein content in wild Black Tilapia (Oreochromis mossambicus)
A. Naji; M. Yousefzadi, University of Hormozgan

Metallothionein (MT) concentrations in soil and liver tissues of *Oreochromis mossambicus* were determined to assess biological response of fish to levels of some metals. Mean concentrations of MT in liver and muscle tissues of 0.052 µg/g dw and 1.85 µg/g dw, respectively, were obtained from 0.01 to 1.0 µg/dw g-1 tissue MT concentration in fish liver and muscle tissues, respectively. The results indicate that MT concentrations were tissue-specific, with the highest levels in the liver. Therefore, the liver can act as a tissue indicator in *O. mossambicus* in the study area.

MO183
Chemical fractionation of Cu and Cd in surface sediments of the southern part of Caspian Sea, Iran
A. Naji, T. Sohrabi, Inland Water Aquaculture Research Institute; M. Yousefzadi, Marine Biology

This study concentrates on the speciation and distribution patterns of some heavy metals (Cu and Cd) in surface sediment samples collected from the Caspian Sea, the biggest lake in the world, to obtain an overall classification for the origins of metals in the area using a sequential extraction technique. All sampling stations, Cu were mostly (>50%) accumulated in the resistant fraction, which indicated that there were no significant anthropogenic inputs of Cu into the surface sediments of the south Caspian Sea. Mazandaran and Golestan provinces in the middle and western parts of the Caspian Sea, in the Iran region were polluted with Cu and Cd. Cd concentrations were in the range of 13.50 to 49.80 µg/g dw, mostly (>50%) accumulated in the resistant fraction, which indicated that there were no significant anthropogenic inputs of Cd into the south Caspian Sea. Cd sulfide pigments are used for artists' paints (PY35, Chromium yellow) and are potentially introduced to sewage treatment plants by cleaning brushes with water. After settling together with the activated sludge in the sedimentation basin, the pigments may be transferred to soils as sewage sludge is often used as fertilizer due to its high nutrient content. It is well known that Cd sulfides are sparingly soluble, however the cadmium can be mobilized by oxidizing or acidic conditions. Mobilized Cd can be transported to the groundwater or be introduced in the human food chain after uptake by plants. Cd is highly toxic, suspected to cause cancer and harmful to the bone structure. For a proper risk assessment it is necessary to determine the mobilization potential of Cd under realistic environmental conditions. In this work column percolation test were used for determination of the mobility of the cadmium pigments under different redox conditions in an accelerated test. For this purpose sewage sludge was spiked with Cd pigment and spiked to soils containing different amount of organic carbon. These soil materials were percolated using artificial rain water. Eluates were sampled over a period of 68 h and in all eluates the pH value, conductivity, turbidity, DOC and Cd-concentration were analyzed. The redox potential was measured in selected columns on-column directly after the outflow. The redox potential is decreasing fast after start of the percolation and shows a clear relation to the organic carbon content of the soil material; eluates of columns with higher organic carbon show a slower decrease in redox potential. In accordance with these results more Cd is released for soils with high organic carbon content.

MO185
Chemical fractionation of Cu and Cd in surface sediments of the southern part of Caspian Sea, Iran
A. Naji, T. Sohrabi, Inland Water Aquaculture Research Institute; M. Yousefzadi, Marine Biology

This work column percolation test were used for determination of the mobilization potential of Cd under realistic environmental conditions. In this work column percolation test were used for determination of the mobilization potential of Cd under realistic environmental conditions. In this work column percolation test were used for determination of the mobilization potential of Cd under realistic environmental conditions. In this work column percolation test were used for determination of the mobilization potential of Cd under realistic environmental conditions. In this work column percolation test were used for determination of the mobilization potential of Cd under realistic environmental conditions. In this work column percolation test were used for determination of the mobilization potential of Cd under realistic environmental conditions. In this work column percolation test were used for determination of the mobilization potential of Cd under realistic environmental conditions. 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MO188

Reduction of chromium toxicity to duckweed Lemma minor in presence of humic acid G. Kakuková, A. Žgajnar Gotvajn, University of Ljubljana / Faculty of Chemistry and Chemical Technology

Humic acid is a major component of humic substances, which are ubiquitous organic matter in the aquatic environment. Humic acids have a great ability to form complexes with heavy metals; they can, under certain conditions, cause abiotic reduction of heavy metals and thereby potentially influence metals’ toxicity. The aim of the present study was to investigate the combined effect of environmentally relevant concentration of humic acid (HA) and chromium (Cr) on toxicity to duckweed Lemma minor in terms of growth rate, chlorophylls and metal content evaluation. In treatment with chromium (Cr = 0.15 mg L\(^{-1}\)) the growth rate, chlorophyll a and b content were reduced by 18%, 51% and 38%, respectively. On the contrary, in the presence of HA (TOC = 10 mg L\(^{-1}\)) all of the investigated parameters stayed comparable to corresponding controls. In the absence of HA, due to the heavy metal almost twice more chromium in comparison to treatments with HA. Analyses of chromium species showed, that in the absence of HA, only hexavalent chromium is present in the medium. However, in the presence of HA most of the hexavalent chromium dissipated from the water phase being reduced to trivalent chromium. The differences in chromium toxicity and accumulation in presence or absence of HA could be explained by diverse mechanisms of both chromium species uptake. Uptake of hexavalent chromium is known to be metabolically driven and carried out by sulphate transporters while trivalent chromium is mainly retained by the cation exchange sites of the cell walls or precipitate on the surface of roots and thus not further transported. Since concentrations of humic acid as low as found in the aquatic environment so significantly altered toxicity and accumulation of chromium (and possibly other heavy metals) its content should be considered as an important factor related to heavy metals toxicity assessment.

MO189

Bioaccessible Nickel in Various Particle Sizes of House Dust from Communities Close to Nickel Mining and Smelting Operations N. Doi, University of Guelph/ St. / School of Environmental Sciences; L. Vasiliuk, B.A. Hale, University of Guelph / School of Environmental Sciences; M. Dutton, Vale Inclo Ltd. Nickel accumulation in mammalian organs after ingestion of elevated levels of nickel from food, water, soil, and dust may be a significant source of exposure for communities close to Nickel mining and smelting operations. Nickel toxicity accumulation can induce chronic inflammation in the liver and kidneys, promoting cancer development in these organs. Measuring sources of nickel exposure for the non-occupationally exposed population are therefore of interest, an example being dust ingestion. Indoor dust particles that settle on surfaces adhere upon contact to the hands of children and adults, who then ingest it through hand-to-mouth behavior. Nickel exposure through dust ingestion needs to be considered to form a separate exposure pathway from soil ingestion, due to higher organic carbon content in dust and its higher total and bioaccessible nickel concentrations.

Bioaccessible nickel, the amount of nickel that becomes available for intestinal absorption as a result of digestion in the stomach, is likely a more accurate exposure measure compared to total nickel concentration present in house dust. Prior work at uncontaminated sites shows that smaller dust particles have higher total nickel concentrations than bulk particles do. For bioaccessible nickel, finer particles can have increased or decreased nickel depending on the site of origin. We are examining the relationship between particle size and nickel bioaccessibility in Port Colborne and Sudbury house dusts from Ontario, Canada. Samples are being separated using plastic sieves into ranges of nitroSBBBC glycine digestion, which simulates stomach pH and churning via shaking the dust with extraction fluid in a water bath at body temperature. Samples will be analyzed using Flame Atomic Absorption Spectrometer. We hypothesize that there is a relationship between particle size and nickel bioaccessibility in house dust, but do not know whether to anticipate that nickel bioaccessibility will be higher or lower in fine particles compared to coarse particles. Further study results will inform risk assessors on the contribution of indoor dust to total nickel exposure through ingestion, for populations close to nickel mining and smelting activities.

MO190

Investigating the concentrations of lead in soil and water next to an abandoned Pb mine in A?jágra (Spain) J. Gala Fernández, University of Extremadura / Unidad de Toxicología Departamento de Sanidad Animal; F. Soler, University of Extremadura / Faculty of Veterinary Medicine; A.O. Jiménez, Unidad de Toxicología Departamento de Sanidad Animal

Environmental pollution from active or abandoned mines can become an important source of metal contamination in soil and water. This study focuses on an abandoned lead (Pb) mine (Las Musas) located in A?jágra (Extremadura, Spain) surrounded by agricultural fields and one nucleus of population. The objectives of this research were to: (a) investigate the pollution distribution of Pb in soils and waters near the Pb mine; and (b) estimate the pollution degree in these environmental matrices on the basis of quality standards for soil and waters. Suitable robust methods of lead determination in soil and water were chosen. Soil samples from soils were taken from 30 cm deep in the soils from the mine (southwest, southeast, northwest, and northeast of the mine for soils and upstream, stream that goes through the mine, and downstream for waters). Pb content was analyzed by anodic stripping voltammetry after acid digestion of the samples. The data showed that the total levels of Pb in soils are not considered of concern since in all sampling sites it concentration not exceeded the regulatory levels established by the regional Environmental legislation of heavy metals in soils from Extremadura for the protection of the human health and ecosystem. On the contrary, Pb concentrations in waters were higher than the regulatory levels established in the environmental quality standards in the field of inland surface waters policy.

MO191

Understanding the chemical speciation of silver from the use of personal care products in aquatic freshwater systems A. Peters, Wca Environment Ltd.; G. Merrington, Environment Agency; S. Lofts, Centre for Ecology & Hydrology / Shore Section; S.D. Smith, Willfrid Laurier University / Department of Chemistry; R. van Egmond; R. Nasr, Willfrid Laurier University / Chemistry

The acute toxicity of silver to aquatic organisms has been extensively tested, and biotic ligand models have been developed for both fish and invertebrates. However when validated there were limitations to their performance. The chronic toxicity of silver has been much less well studied, and the factors modifying bioavailability (i.e. interactions with complexing ligands) are not sufficiently well understood to enable robust models to develop. Silver is known to form strong complexes with both Dissolved Organic Matter (DOM, e.g. humic and fulvic acids), reduced sulfide ligands (e.g. free sulfide S\(^{-2}\)), and also with thiol compounds (e.g. cysteine). Attempts to understand the speciation of silver in ecotoxicity tests have revealed significant limitations in our ability to predict silver speciation under different water chemistry conditions. The relative importance of the different strong silver-complexing ligands is uncertain and the uncertainties regarding their concentrations in natural systems, means that a meaningful interpretation of the existing chronic ecotoxicity data for silver in terms of biotic ligand model approaches is not currently possible. The reasons for these uncertainties, and the approaches which may be taken to address them, will be outlined. The key aims of this study are to determine silver speciation in a range of aqueous systems, from simple well defined systems with individual ligands to complex systems with multiple ligands. This information is being used to determine how well WHAM VII predicts Ag complexation in receiving waters in relation to variable DOM-sulfide and free sulfide concentrations. There are differences in the binding of silver by DOM from different sources which cannot currently be explained by WHAM, but appear to depend on the DOM source. Some DOM species bind sulfide ligands and their silver complexes are explicitly included in model calculations. Whilst it is clear that dissolved silver can be highly toxic to some aquatic organisms, a better understanding of the conditions which are likely to result in toxicity to organisms and ecosystems would lead to considerable advances in understanding the potential risks from silver in surface waters.

MO192

A meta-analysis for assessing the relationship between metal toxicity and metal affinity for binding to the biotic ligands of soil and aquatic organisms M.M. Ardestani, Ecological Science; N.M. van Straalen, VU University Amsterdam / Department of Ecological Science; C.A. Van Gestel, Vrije Universiteit; Department of Animal Ecology

Biotic ligand models (BLMs) have been developed to predict the toxicity of different metals to different organisms, while work on terrestrial organisms is still in progress. A BLM simulates stomach pH and churn ing via shaking the dust with extraction fluid in a water bath at body temperature. Samples will be analyzed using Flame Atomic Absorption Spectrometer. We hypothesize that there is a relationship between particle size and nickel bioaccessibility in house dust, but do not know whether to anticipate that nickel bioaccessibility will be higher or lower in fine particles compared to coarse particles. Further study results will inform risk assessors on the contribution of indoor dust to total nickel exposure through ingestion, for populations close to nickel mining and smelting activities.
well as other organic and inorganic ligands, competing or making complexes with the free metal ions to bind to the biotic ligands. The main assumption of these models is that when an organism is exposed to metal in different media, only a fraction of the total metal load is bound to the site of action (biotic ligand) in the organism and as a consequence adverse effects occur. Each metal has a specific affinity for binding to the biotic ligands of an organism which is qualified by the conditional binding constants. In this study the relationships between metal toxicity and metal affinities for binding to the biotic ligands of soil and aquatic organisms were investigated. We used data from the literature on terrestrial organisms such as plants, earthworms, and collembolans and on aquatic species such as fish and invertebrates as well as other organisms like bacteria and fungi. We distinguished the conditional binding constants derived from toxicity or bioaccumulation experiments. In such cases, toxicity and well correlated with the biotic ligand binding constants. Considering the effect of competing cations, major ions such as protons, calcium, and magnesium had the most significant effects on metal toxicity and as a consequence on the relationships between toxicity and strength of metals for binding to the biotic ligands. The correlations between toxicity and binding constants were obtained for single test species exposed to different metals and for all species together. Toxicity of Ag and Cu was highest for most test species. For almost all organisms, conditional binding constants for Ag, Cu, and Cd were higher than those for Zn and Ni. The values derived for aquatic systems seem to be equally valid for soil organisms, but in the case of soils, bioavailability from the soil solution is greatly influenced by the presence of the soil solid phase.

MO193
An experimental system representative of groundwater hydraulic conditions to test the applicability of diffusive gradients in thin film (DGT) for groundwater monitoring
C. MORTON / Consultant Division; S. Robert, Suez Environment / CIREE; J. esthem, BRGM / Laboratory Division; T. Lamonux, L. Amarite, BRGM; A. Bruchet, CIRSE Suez Environment
Key words: diffusive gradients in thin film (DGT); groundwater, diffusive boundary layer (DBL), experimental system The validated common approach of groundwater monitoring is based on spot sampling after pumping followed by laboratory analysis but has well-known drawbacks (formation of contaminant distribution between the whole aquifer and groundwater sampled by pumping homogenization of the water column which can hide a potential stratification of contaminants ...). Passive sampling techniques present several advantages by comparison to the classical sampling approach (they neither need pumping, nor disturb the groundwater). However, few publications deal with the use of innovative passive sampling methods. In this study, the aim was to test the applicability of diffusive gradients in thin film (DGT) for metal monitoring in groundwater (Pb, Cd). Indeed, a diffusive boundary layer (DBL) forming on DGT surface especially in case of low flow conditions influences the uptake of metals. First of all, an experimental system representative of groundwater hydraulic conditions was developed. The system is composed of three open columns with an ascending water circulation of a natural groundwater spiked with the targeted compounds (Pb, Cd, Pb). The flow is respectively in each column of 1, 2 and 4 m/day. DGT with several diffusive gels thicknesses were deployed in triplicates during 7, 14 and 21 days in each column. During the experiments, the concentration of metals in water and the uptake of compounds were followed to verify the linear accumulation with time. The DBL was estimated at 13 and 21 da for Pb and Cd, lower than those found in literature were found. By taking them into account, TWA concentrations were found close to concentration of Pb and Cd measured in water samples. We acknowledge financial support from French National Research Agency (ANR ECOTECH 2011 ORIGAMI project 11 ECOT 003)

MO194
Abandoned mine legacies: Determining the extent and impacts of pollution via a case study in the South West England
S. Comber, Plymouth University / Environmental Science; S. Beane, Plymouth University / Environmental Science; C. Wood, Plymouth University / Geography, Earth and Environmental Sciences; P. Long, Environment Agency
For parts of the UK and other European countries, contaminated land resulting from millennia of mining activities are contributing significantly to failures in water quality for a number of metals including lead, nickel, zinc, copper and cadmium under the Water Framework Directive. Practicalities such as land ownership, access to diffusive gradients in thin film experiments and implementing remediation options complex and potentially costly. In order to successfully apply any measures, it is necessary to both fully understand the spatial extent of the contamination (including diffuse inputs from soil, spoil and mineral processing areas as well as obvious point sources from adits), the potential impact the metals are having on the receiving water chemistry in terms of speciation and hence potential negative ecological impacts. This paper reports on a specific case study in the SW of England (River Tavy catchment) where the extent of metal contamination around an abandoned mine site has been established in concert with assessment of metal bioavailability and impacts on receiving waters with the objective of supporting planning for future remediation.

MO195
Dissolved Organic Carbon (DOC) Characteristics in Metal-rich Waters and Implications for Copper Aquatic Toxicity
K.T. Lee, Geochemistry; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry
Free copper (Cu++) is a well-known contributor to heavy metal toxicity in aquatic systems. The concentration and bioavailability of Cu++ is influenced by aqueous complexation, with humic and fulvic acids being especially important ligands. This research focused on examining changes in the binding affinity of fulvic acid (FA) that result from chemical fractionation occurring during the formation of hydrous iron and aluminum oxides (HFO and HA0) in AMD/ARD impacted streams. The unaltered and DOC remained column was used to simulate the binding affinity than DOC in aquatic systems without the presence of HFO and HA0. Free copper concentrations were measured with a cuvric ion specific electrode (ISE). FA's used in this study were collected from three arpine watersheds in Central Colorado that showed variability in spectroscopic properties of SUVA254 and fluorescence index (FI) to correlate with aromaticity and DOC source. The binding affinity of DOC is related to its inherent aromaticity and a for lower SUVA254 (lower aromaticity) suggest a lower binding capacity of DOC and a resulting higher concentration of Cu++ in solution. This variation in DOC-Cu binding affinity is likely a significant factor in copper toxicity in aquatic systems and in toxicological modeling programs such as the Biotic Ligand Model (BLM).

MO196
Accumulation of metals by Ambystoma mexicanum in an urban wetland: theoretical and experimental correlation with ambient metal content
C. Ponce de Leon, Facultad de Ciencias; S. Rendón, G. Aldana, Universidad Nacional Autonoma de Mexico / Facultad de Ciencias; M. Hernández, Facultad de Ciencias UNAM / Biology; C. Vanegas, UNAM, Mexico City / Ecology and Natural Resources
All organisms require a variety of essential metals for their metabolic functions whose concentrations are homeostatically regulated. At the same time, organisms possess regulatory mechanisms that prevent and control the accumulation and potential toxic effects of metals, essential and without known biological function. The bioaccumulation of metals depending greatly on the chemical metal specie in the environment interacting with the organism, whilst the bioavailable concentration of these chemical species depends on a variety of physicochemical parameters such as organic matter, clay content, pH, salinity, redox potential, etc., thus making it difficult to predict the organism’s metal accumulation. In this study we assess the physicochemical parameters and metal concentration in sediments in a peri-urban wetland located south of Mexico City. In situ metal accumulation by juvenile Ambystoma mexicanum was assessed in selected sites from the above mentioned study and correlated with water and sediment metal concentrations as well as with theoretical bioavailable metal (Free metal) from water with an equilibrium speciation model for waters, the Windermere Humic Aquatic Model (WHAM). The results showed that the highest concentrations detected in the axolotl liver were the essential metals. However, the concentration of several metals without known biological function (i.e. cadmium, lead) are similar or higher to those reported as critical levels for aquatic life. The free metal WHAM modeling simulating diffusion had limited correspondence with liver metal accumulation in the organisms, rather, sediment metal concentration more accurately reflected the metal accumulation in the organism, suggesting ingestion as the main route. Further WHAM simulations are offered as proposed by Stockdale et al. (2010) in Aquatic Toxicology. Track and sessions: “Metals in the environment: Fate, speciation and bioavailability in water, soil and sediment” in “Analysis, fate and behavior of contaminants”. (Co-authors: Claudia PONCE DE LEON HILL, Sherezada GONZÁLEZ RENDON, Manuel HERNANDEZ QUIROZ, Giovanni ALDANA and Cecilia VÁNEZAS PÉREZ. Unidad de Análisis Ambiental, Facultad de Ciencias, UNAM, Mexico City. Key words: metals, bioavailability, WHAM, Ambystoma mexicanum Presentation preference: Platform

MO197
Comparison of four pore water sampling methods for metals and dissolved organic carbon
B.G. Brumbaugh, US Geological Survey / Columbia Environmental Research Center; D. Cleveland, National Institute of Standards and Technology; J.M. Besser, USGS- Biological Resources Division / Columbia Environmental Research Center; D.D. MacDonald, MacDonald Environmental Sciences Ltd.
Toxicity of metals to aquatic invertebrates is most often attributed to bioavailable fractions of dissolved metals in the interstitial (pore) water of sediments. Bioavailability for many toxic metals is known to depend greatly on certain water quality factors, especially hardness and dissolved organic carbon (DOC). Several pore water sampling methods have been investigated, but recommendations for individual methods vary. Importantly, there have been few, if any studies that compared sampling methods for both DOC and metals, and especially mixtures of metals. Our study compared four different methods for sampling pore water including: peepers, diffusion gradient in thin-film (DGT) probes, centrifugation and filtration, and push-point suction and filtration. Three field-collected sediments with elevated concentrations of metals representing different levels of organic matter, acid-volatile sulphide, and grain size were evaluated, both unsupplied and
spiked. Spiking included cadmium, copper, nickel, lead, and zinc added at amounts in proportion to individual probable effect concentrations (PECs) and anticipated relative sediment binding capacities. Results of these comparisons will be presented and strengths and limitations of each of these methods will be discussed.

MO198 Mechanisms of nickel toxicity in freshwater organisms: A practical approach for supporting mechanistic-based risk assessment tools
K.V. Brix, University of British Columbia / Zoology; C.E. Schlekat, NiPERA; E.R. Garman, NiPERA / Ecotoxicologist

Assessment of ecological risk of nickel (Ni) uses mechanistic-based, albeit empirically derived, predictive tools such as Biotic Ligand Models (BLMs). Despite a number of detailed studies, the precise mechanisms of chronic Ni toxicity to aquatic organisms remains elusive. A workshop was held in November 2014 to review currently available information on mechanisms of Ni toxicity to aquatic organisms. The goal of the workshop was to provide recommendations that can be used in future studies to build on existing mechanistic information. Freshwater aquatic organisms were the focus of discussion because of their sensitivities to Ni, the relatively abundant amount of mechanistic information available, and their importance in risk-based regulations like the Water Framework Directive and REACH. Evidence exists in the literature to support a number of possible mechanisms, including disruption of trace element homeostasis (e.g., Ca, Mg, and Fe), disruption of energy metabolism, and oxidative stress through the creation of reactive oxygen species. Workshop participants recommended using an adverse outcomes approach that includes releases to copper roofs during storm events. A copper roof runoff tool is in development for use by regulators and other stakeholders to quantify the potential ecological impacts of copper roof runoff in receiving waters. It considers three key components of copper runoff fate and transport: 1) copper emission from roofs during rain events; 2) copper attenuation in runoff prior to reaching receiving waters; and 3) copper bioavailability and fate in receiving waters. This study examined attenuation of copper from roof runoff and the factors influencing copper bioavailability and toxicity in a stormwater treatment system. Both laboratory bioassays and modeling with the biotic ligand model (BLM) were utilized. A 3 x 6 meter copper roof structure was built along with two biofiltration swales and two bioretention planters. Runoff samples were collected monthly from the roof downspouts and from the outlets of the swales and planter boxes. SCM copper removals were 82.6 – 99.2% (plants) and 93.1 – 99.3% (swales) by concentration. For a subset of storms, toxicity tests of stormwater were performed with Daphnia magna neonates. Stormwater toxicity testing can result in poor survival due to the low ionic strength of runoff that has not interacted substantially with soils or mixed with surface water. Utilizing cultures adapted to low hardness results in greater survival in reference stormwater. In toxicity tests using these adapted cultures, no survival was observed in copper roof runoff samples taken from the downspout (i.e., SCM influent). Survival from the planter box and swale outlets was ~86% and ~95%, respectively. Modeled D. magna LC50 values for the influent averaged 8 μg/L, while the swale and planter box outlets averaged 180 μg/L. This indicates a decrease in copper bioavailability and potential toxicity as water passed through the biofilter and planter boxes. Modeled toxicity was found to be strongly dependent on pH. This is critical since measured pH values in low ionic strength stormwater can vary based on the technique used. These results indicate that the swales and planter boxes can improve water quality prior to stormwater encountering biological receptors. These data, when integrated into the copper roof runoff tool, will allow more accurate assessment of potential impacts in receiving waters.

MO200 Effects of wildfires on metals mobilisation in eucalypt and pine forests
J. Campos, Universidade de Aveiro / Department of Environment; N. Abrantes, University of Aveiro / CESAMDAO; C. Vale, IPMA / Instituto Portugueses do Mar e da Atmosfera; P. Pereira, Department of Biology and CESAM; J. Keizer, Department of Environmental Centre for Environmental and Marine Studies CESAM University of Aveiro

Aveiro Portugal

Forest fires are increasingly frequent and severe in Mediterranean region, mainly in Portugal, with huge environmental impacts. Among all the effects of fire on soil properties, deposition, mobilisation and fate of contaminants, with particular reference to trace metals, has been a relatively neglected issue. In fact, wildfires can release and deposited metals on soil surface, either directly by combustion of vegetation and soil organic matter mineralization or via re-availability from ashes–soil interactions. These contaminations are of major concern due to their high toxicity, environmental persistence and tendency to bioaccumulate in the food chain. Furthermore, these contaminants find their way into groundwater and surface sources via leaching and or into aquatic systems by overland flow (runoff) that promotes the inputs of ash and eroded particles of soil in water. The main objective of this study was to evaluate the input of metals (Mn, V, Co, Ni, Cu, Cd and Pb) in soils and ashes of eucalypt and pine forests (Ermoia, North-centre of Portugal) associated with a wildfire and followed by rainfall events. This was accomplished by monitoring the temporal evolution (immediately after the fire, 4-, 8- and 15-months after the fire) of metals in soils and ashes. Concentrations of V, Mn, Co, Ni, Cu, As, Cd and Pb were analysed by inductively coupled plasma mass spectrometry (ICP–MS), after an acid digestion. The principal results obtained in this study were the following: (1) The levels of Mn, Pb, V, Ni and Cd were higher in the burnt soils compared with those in the non-burnt soil, while for As the opposite trend was observed. As for Cu and Co no differences were found; (2) No temporal differences were found for metals concentrations in the non-burnt soils; (3) Metals levels in burnt soils differed for the four sampling occasions but not in straightforward manners; (4) Manganese, Pb, V, Cu, Ni, Co and Cd have peaked in ashes collected immediately after the fire, while the opposite was observed for As; (5) In general, metals levels (with the exception of As) declined sharply during the first 4 months and remained unchanged 8 and 15 months after the fire. Our results highlight that wildfires play a key role in the mobilisation of metals in the environment and underline the importance of further studies about the associated risks in terms of ecotoxicological effects, both in-situ and in downstream aquatic systems. Keywords: Wildfire; Metals; Soils; Ashes

MO201 HEAVY METAL POLLUTION BY URBAN SUPERFICIAL RUNOFF: ACCUMULATED SEDIMENTS ON SOACA-BOGOTA ROAD (COLOMBIA)
N.A. Peña, IRTA; S. Alvarez, C.A. Zafra, Universidad Distrital Francisco José de Caldas / Faculty of Environmental Development Program; K.J. Rader, Mutch Associates, LLC; J.W. Gorsuch, Copper Development Association Inc.; R.E. Casey, Towson University / Chemistry

Concerns have been raised by regulators over diffuse and nonpoint sources of metals including releases from copper roofs during storm events. A copper rooftop runoff tool, will allow more accurate assessment of potential impacts in receiving waters. A 3 x 6 meter copper roof structure was built along with two biofiltration swales and two bioretention planters. Runoff samples were collected monthly from the roof downspouts and from the outlets of the swales and planter boxes. SCM copper removals were 82.6 – 99.2% (plants) and 93.1 – 99.3% (swales) by concentration. For a subset of storms, toxicity tests of stormwater were performed with Daphnia magna neonates. Stormwater toxicity testing can result in poor survival due to the low ionic strength of runoff that has not interacted substantially with soils or mixed with surface water. Utilizing cultures adapted to low hardness results in greater survival in reference stormwater. In toxicity tests using these adapted cultures, no survival was observed in copper roof runoff samples taken from the downspout (i.e., SCM influent). Survival from the planter box and swale outlets was ~86% and ~95%, respectively. Modeled D. magna LC50 values for the influent averaged 8 μg/L, while the swale and planter box outlets averaged 180 μg/L. This indicates a decrease in copper bioavailability and potential toxicity as water passed through the biofilter and planter boxes. Modeled toxicity was found to be strongly dependent on pH. This is critical since measured pH values in low ionic strength stormwater can vary based on the technique used. These results indicate that the swales and planter boxes can improve water quality prior to stormwater encountering biological receptors. These data, when integrated into the copper roof runoff tool, will allow more accurate assessment of potential impacts in receiving waters.

MO202 Modeling the Fate of Metal Concentrates Using the TICKET Unit World Model
K.J. Rader, Mutch Associates, LLC; R.F. Carbonaro, Mutch Associates, LLC / Civil and Environmental Engineering; D. Delahay, European Copper Institute; K.J. Farley, Manhattan College / Civil and Environmental Engineering

Among the key challenges associated with metal concentrates assessment and classification are their sparingly soluble nature and their long term following release. A concentrate's solubility affects its bioavailability. The environmental fate of a substance is in part described by its degradability. While metals do not "degrade" according the classic definition typically applied to organics, their solubility affects their capacity to cause adverse effects in the environment is not without temporal limits. Assessment of metal "degradability" is not as straightforward as for organics where resources were transported by runoff and deteriorates the air quality when suspended by wind and the turbulence induced by traffic. This study presents the results of a pollution assessment on heavy metal particles associated to road sediment from two particular areas (i.e. Zones 1 and 2) of the Bogotá-Soacha highway (Colombia). Road sediments were collected in each area by direct sweep over a surface of 0.25 m² and for a period of four months. The concentration of Mn, Fe, Cu, Zn, As, Cd, Ba and Pb was determined per size fraction (less than 250 μm from dry road sediment). These figures were obtained by means of flame atomic absorption spectrometry and also by using inductively coupled plasma mass spectrometry ICP–MS. The results suggest that the main origin of heavy metals was from road sources such as tarmacs, but not in traditional ways. In addition, it is shown that the concentration of Pb and Zn was higher than that allowed by current regulations (used as reference). With regard to urban soils, heavy metals that exceeded the permissible limits were as follows: Pb (21.7% and 87.0% of the time in Zones 1 and 2, respectively) and Zn (84.8% and 82.6% of the time in Zones 1 and 2, respectively).

MO203 MODELING THE FATE OF METAL CONCENTRATES USING THE TICKET UNIT WORLD MODEL
K.J. Rader, Mutch Associates, LLC; R.F. Carbonaro, Mutch Associates, LLC / Civil and Environmental Engineering; D. Delahay, European Copper Institute; K.J. Farley, Manhattan College / Civil and Environmental Engineering

Among the key challenges associated with metal concentrates assessment and classification are their sparingly soluble nature and their long term following release. A concentrate's solubility affects its bioavailability. The environmental fate of a substance is in part described by its degradability. While metals do not "degrade" according the classic definition typically applied to organics, their solubility affects their capacity to cause adverse effects in the environment is not without temporal limits. Assessment of metal "degradability" is not as straightforward as for organics where standardized test procedures are available. Nevertheless, the implications of the degradability criterion for metals are significant particularly when the concentrates are part of maritime cargoes subject to MARPOL Annex V requirements. Based on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS), metals are classified as "degradables" if they are one of the 31 prioritized metals, or their "degradability" of soluble metals was proposed in a 2011 European Union Classification, Labelling and Packaging (EU CLP) draft guidance document. In analogy to organic chemicals, “rapid degradation” for metals requires greater than 70% removal within 28 days. However, unlike organics, where removal from the
water column occurs via degradation, metal removal occurs through changes in speciation (partitioning and precipitation) followed by sedimentation which transfers metal to the sediment. Therefore, “rapid degradation” for metals requires one to demonstrate rapid and functionally irreversible loss from the water column. This entails 1) sorption of dissolved metal to suspended particles or direct precipitation, 2) transport of particulate metal to the sediment, and 3) functionally irreversible uptake of metal in the sediment through precipitation and sorption. A unit world model for metals in lakes, TICKET-UWM, was applied to the assessment of concentrate bioavailability and degradability in a generalized water body. Model results indicate that settling of concentrates limits the direct impact dissolution had on water column metal concentrations. Continued dissolution of concentrates in the sediment resulted in some recycling of metal to the water column. Therefore, water column metal concentrations resulting from this sediment flux was very low. The lack of significant remobilization indicated by the model is generally consistent with data from field and laboratory studies.

MO203 Sediment Cu bioavailability – the use of diffusive gradient thin films (DGT) and effects of chitosan amendment
T. Oksanen, University of Eastern Finland / Department of Biology; K.K. Vaananen, University of Eastern Finland / Biology; J. Akkanen, University of Eastern Finland / Department of Biology
Mining industry often increases metal loading in surrounding environment. In water ecosystems, a part of the metals is stored in sediment compartment. When environmental conditions change, metals may be released back to water causing toxicity. Due to these fluctuations, a risk assessment for metals is challenging, and widely used sediment quality guidelines are often inadequate for site-specific risk assessment. We used diffusive gradient thin films (DGT) a) to observe bioavailable fraction of Cu in sediment, b) to study metal bioavailability reducing effects of chitosan amendment in Cu-spiked natural sediment and c) did toxicity tests to reduce possible toxicity of Cu, as well as its mobility and/or chitosan (L. variegatus). Natural lake sediment (Parkkimäjärvi) in Finland was chosen for this study. ICP-MS was used for metal concentration analysis. Possible toxicity was tested with 28-day reproduction and growth experiment using L. variegatus. Sediment was spiked with 25-410 mg/kg dw Cu and with 0, 0.5 and 5 % dw chitosan. In first experiment, DGT concentrations correlated with total copper concentrations. It indicates that in this sediment, DGT can be used for determining reactive concentrations of Cu. Chitosan adsorbed bioavailable copper (410 mg/kg dw) in chitosan concentrations of 0.5 (32 % reduction) and 5 % (87 % reduction). Chitosan had adverse effects on weight of L. variegatus, but not on reproduction. Higher Cu concentrations had adverse effects on total biomass, but no statistical difference was seen in reproduction or single worm weight at their own. Sediment ingestion is the main Cu-uptake route for L. variegatus, but the reduction of active Cu shown in DGT-experiment was not that clear according to toxicity test. DGT seems to be a promising tool for assessing metal bioavailability and risks in sediments. Chitosan was effective in binding copper in slightly acid sediment (pH 5-6). On the other hand, adverse effects of chitosan were seen in toxicity test. As a result, further studies should be concluded for the use of chitosan in remediation.

MO204 Risk assessment of the use of organic wastes as soil amendments: results from a field experiment
P. Álvarez-Castillo, Polytechnic Institute of Beja / Department of Technologies and Applied Sciences; M. Farto, Polytechnic Institute of Beja / Department of Technologies and Applied Sciences; M. Farto, C. Mourinha, I. Dones, M. Patanita, Polytechnic Institute of Beja; J. Renaud, Department of Life Sciences; T. Natal-da-Luz; J. Sousa, University of Coimbra / Department of Life Sciences/CEFED.
The use of organic wastes as soil improvers seems as an attractive option in soil amendment, because it would enable valuable components to be recycled (e.g. organic matter, N, P, K). However, this practice represents a potential risk to the environment, because of probable high heavy metal content of the wastes, a problem that may be aggravated if the metals are mobilized in the soil, available for plant uptake or transported in drainage waters. Taking this in account, a field experiment was established with different organic wastes: sewage sludge (SS), manure (Ma), compost (Com), and compost produced from agricultural wastes (CAW), at 6, 12 and 24 ton dry matter ha⁻¹, with Lolium perenne L., in order to assess the behaviour of metals in the soil/plant system and the effects of the amendments in soil chemical and biochemical properties. The SS application had a beneficial effect on soil production (chlorophyll, foliar area, biomass) and on soil properties (organic matter content, total nitrogen, available phosphorus, and cation exchange capacity). The effect of the two amendments on both composts application, without a significant increase in total heavy metals concentration, both in soil and in plants. Dehydrogenase activity was also positively affected by the amendments application. Accumulation factors for heavy metals in plants were low, and their concentrations in the plant were lower than the maximum tolerable level for cattle, used as an indicator of risk of entry of those metals into the human food chain. Two different heavy metal extractable fractions were determined: a) mobile fraction, and a mobilizable fraction, which will allow a deeper discussion on the fate and behaviour of heavy metals in soils, and on the risk of the use of these organic wastes as soil amendments.

MO205 Contrasting abilities of metal bioaccumulation in Gammarus populations with different exposure histories
N. Uiren, J.D. Lebrun, Iseroa; A. Farafara, Iseroa Antony; a. chaumont, Iseroa / UR MALY Laboratoire Ecotoxicologie; L.C. Fechner, Iseroa Antony / UR HBAN; O. Geffard, Iseroa / UR MALY Laboratoire Ecotoxicologie
Gammarus are net accumulators of metals, hence considered as integrated indicators of metal bioavailability in freshwater. However, aquatic organisms live in contrasted systems in terms of water chemistry and anthropic contaminations, including metals, to which they are acclimated. Indeed, chronic exposure may induce physiological mechanisms likely to modulate metal regulation (e.g. metallothioneins synthesis), thus bioaccumulation. So, differences in exposure histories of populations may lead to differences in accumulation patterns. In gammarids, tolerance to metal exposures is few documented and need to be evaluated conditioning the relevance of using gammarids to monitor water quality. The use of biodynamic modelling to describe metal bioaccumulation is now well established. Biodynamics assumes that bioaccumulation is the result of a balance between uptake and loss rates of contaminant, characterised by specific kinetics parameters for each metal. Here, we propose to use the comparison of kinetics parameters as approach to detect differences between populations and therefore to identify which of the physiological parameters is modulated (uptake and/or loss). In our previous studies, the between-population variation of kinetics parameters controlling bioaccumulation in several unexposed (control) populations of Gammarus has been defined and is proposed in this study as reference. The aim of this study was to investigate the metal bioaccumulation abilities of two different naturally populations of gammarids pre-exposed to metals in their natural environment and then to compare their kinetics parameters to values previously obtained with controls populations. To this end, three Gammarus populations, pre-exposed to Pb or Cd or Pb/Ni contamination, were collected and then exposed, in laboratory conditions, to various metal concentrations (Pb, Cd and Ni) for seven days immediately followed by a seven days depuration period. The internal metal concentrations in gammarids were measured over time. Then, kinetics parameters of metal describing bioaccumulation were determined for each population. Finally, results will inform whether pre-exposure of populations may modulate their bioaccumulation capacities and the occurrence of potential tolerance mechanisms.

MO206 Comparison of in vitro estimations of bioaccessible Ni in field-contaminated soils and identification of mineralogy
L. Vasilik, University of Guelph / School of Environmental Sciences; J. Wragg, M. Cave, British Geological Survey; M.D. Dutton, Vale INCO; B.A. Hale, University of Guelph / School of Environmental Sciences
The bioavailability of contaminants in sediments depends on their specific properties. The bioaccessibility of a contaminant mixed with the soil matrix depends upon the soil properties, the contaminant and the manner by which the contaminant entered the soil. The study considers soil Ni contamination around smelting and refining sites, such as the Vale complex in Sudbury (SUD) and Port Colborne (PC), ON, Canada. The objective was to determine if there was a relationship among particle size, metal concentrations and metal bioaccessibility. At each location, two naturally different populations of gammarids pre-exposed to metals in their natural environment and then to compare their kinetics parameters to values previously obtained with controls populations. To this end, three Gammarus populations, pre-exposed to Pb or Cd or Pb/Ni contamination, were collected and then exposed, in laboratory conditions, to various metal concentrations (Pb, Cd and Ni) for seven days immediately followed by a seven days depuration period. The internal metal concentrations in gammarids were measured over time. Then, kinetics parameters of metal describing bioaccumulation were determined for each population. Finally, results will inform whether pre-exposure of populations may modulate their bioaccumulation capacities and the occurrence of potential tolerance mechanisms.

J.M. Blais, A. Houben, University of Ottawa; J. Korosi, A.J. Poulin, L. Kimpe, University of Ottawa / Biology
This project is to generate information on the extent and fate of environmental contamination. This includes developing new tools, such as diffusive gradient thin film (DGT) and chitosan, to assess the bioavailability of metals in sediments and soils. Additionally, the use of bioaccumulation models to predict metal bioaccumulation in aquatic organisms is also being explored. The project will provide valuable insights into the ecological consequences of legacy pollutants in freshwater systems.
The activity of peroxidase (POD) was increased in plants treated with different concentrations of NaCl. Increased POD activity might be a plant mechanism of alleviation of oxidative damage induced by salt. Different concentrations of Cd in the medium and a combination of Cd and NaCl had no statistically significant effect on POD activity. There was no statistically significant effect of cadmium, sodium chloride, or interaction of these factors on total protein concentration in plant material.

MO210
Rainfall erosivity index E130 and losses of Cu-based fungicide in vines via throughfall
P. P. Rodríguez, Universidade de Vigo / Plant Biology and Soil Science; D. E. Gomez, The University of Exeter / College of Engineering Mathematics and Physics; P. Cancelo, University of Santiago de Compostela; M.Paraudeau, I.L. Periago, University of Vigo / Plant Biology and Soil Science

Cu-based fungicides are widely applied in vineyards to fight against fungal diseases. Cu losses by rainfall may lead to an enrichment of Cu in soils becoming an important soil pollutant and even may reach nearby basins. Rainfall energy may influence Cu losses although it has never been studied before in vineyards. The objectives of this study are: 1) to quantify Cu losses under natural rainfall and 2) to examine the influence of rainfall erosivity index E130 (Wischmeier & Smith 1978) on Cu losses. Two nearby vineyards in Ribadavia (Galicia, Spain) were monitored during crop years 2009 and 2010. Collecting rainfall and throughfall up to 16 trays were placed under the vines to maximize the collection of the throughfall. Soluble-Cu fractions (CuS) in the water fraction < 0.45 micron ii) the Particulate-Cu fraction (CuP) were collected from trays after each rainfall episode. Additionally, daily E130 values (M ha⁻¹ mm h⁻¹) for the same period were calculated from a tipping bucket pluviometer 5 min. Absolute frequency distributions of throughfall volume in collecting trays(mm) and throughfall/rainfall ratio are bimodal. Small rainfall episodes brought more less throughfall. Higher rainfall episodes gave more throughfall/rainfall ratio as expected. Throughfall average in all episodes was 14±17% of rainfall being 55% the maximum. That was 14.9±15.7 mm ranged between 0.3 and 44.4 mm. Concentration of CuS in throughfall in relation to accumulated E130 per sampling is higher at the beginning of the experiment, decreasing sharply with subsequent rainfall episodes. CuP is expressed as mg Cu per kg of total solids in throughfall samples. Solids result from the rainfall wash-off of autochthonous fungal sprays and natural dry deposition on leaves. CuP is about three orders of magnitude higher than CuS giving an average Solid/liquid distribution ratio above 1000 L/kg. The highest concentrations were also found in the first rainfall event although CuP and these data are fitted to a hyper-exponential model, which explains that CuP losses happened in two ways: one quickest than the other where bigger and stacked particles are detached firstly. Finally, Cu loss is dependent on rainfall erosivity index EI30 due to the lack of leaves and the very first rainfall episodes. Wischmeier, W.H. y Smith, D.D. 1978. Predicting Erosion Losses. Agriculture Handbook 537. United States Department of Agriculture. Science and Education Administration. 58 pp.
MO212

Particle elements and element speciation in seafood samples from different contaminated sites in Europe

A. Maunula, IPMA, I.P. / Division of Aquaculture and Seafood Upgrading; P. Anacleto, V. Barbosa, IPMA / Portuguese Institute for the Sea and Atmosphere / Division of Aquaculture and Seafood Upgrading; J. Slotj, R. Rasmussen, K. Gedde, DTU - Technical University of Denmark; M. Fernandez-Tadeso, AEIFORIA; F. Heuvel, Hortinmare, M. Kotteman, IMARES / Fish; A.M. Marques, Portuguese Institute for the Sea and Atmosphere / Division of Aquaculture and Upgrading

Chemical pollution of aquatic ecosystems represents an ecological threat that has been receiving increasing attention. In environmental monitoring, marine biota from different trophic levels (e.g., algae, bivalves, and fish species) are commonly used to assess the levels of chemical contamination, accounting for their propensity to accumulate certain contaminants. Coastal areas exposed to high environmental contamination by one or more pollutants from anthropogenic or natural sources are called "hotspots", being generally associated with highly urbanized and industrialized areas. Toxic elements (e.g., Hg, As, Cd and Pb) are widely present in the aquatic environment and may reach high levels in these hotspots. However, the elements' toxicity depends on the chemical form and oxidation status, which influences their bioavailability, transport, persistence and impact in the food chain. Thus, assessing the speciation of some compounds, such as Hg and As, is also highly relevant from an ecological and food safety point of view. In this context, the present study aims to evaluate the bioaccumulation of toxic chemical elements (Hg, As, Cd, and Pb) in bivalves and fish species, considering factors such as biomass, sex, and food habits. Moreover, data of mean diameter obtained through laser diffraction (LDS) and field emission-scanning electron microscopy (FESEM) was used to estimate the size distribution of E1 and E3 according to obtained by SEM. Moreover, data of mean diameter obtained by Dynamic light scattering (DLS) was used to estimate the size distribution of E1 and E3, as suspensions that can be dehydrated by rainfall wash-off from vines sprayed with agrochemicals. Additionally, throughfall samples were examined by SEM taken with an environmental scanning electron microscope (ESEM) equipped with a field emission gun (FEI Quanta 200, Netherlands). Results obtained show that Co comprises polydispersity population noting that bigger particles are located on the top of the stack while smaller ones are in the bottom by drying drop with several particles sizes ranged 20 to 2000 nm. Additionally, throughfall samples analyzed by DLS show that polydispersity index (PDI) calculated according to the Cumulants method was 0.454 for E1 and 0.350 for E3 according to obtained by SEM. Moreover, data of mean diameter obtained as well as size distribution of E1 and E3 suggest that bigger particles are detached first whereas smaller ones are washed after the third rainfall event. Finally, Cu substances, rain dry deposition and plant exudates as well as fungi, bacteria and spores can be present in the throughfall.

MO215

Temporal trends of heavy metal concentrations in Arctic Char (Salvelinus alpinus) from an Austrian High Mountain Lake: effects of climate-induced changes in lake water chemistry

G. Köck, Institute for Interdisciplinary Mountain Studies; D.C. Muir, Environment Canada / Aquatic Contaminants Research Division; R. Lackner, R. Tessadri, University of Innsbruck; K. Koning, Innsbruck University

Due to similar environmental characteristics (e.g. long ice-cover, oligotrophy) high-altitude and high-latitude lakes are very sensitive ecosystems where even small environmental changes (e.g. input of pollutants, climate change) may significantly affect aquatic ecosystems. The Austrian High Mountain Lake cooperation High-Arctic 1997-2014 is investigating the effects of short-term and long-term climate change on freshwater ecosystems in the Austrian Alps and in the Canadian High Arctic. Studies from the 1990s on Arctic char (Salvelinus alpinus) from Schwarzeee ob Sölden (SO), the highest fish-bearing lake in the Alps (2792 m.a.s.l., Ortal Valley, Austria), revealed extremely metal contamination of this fish. Levels of cadmium (Cd) and lead (Pb) in liver and kidney tissues were comparable to those of fish from waters receiving metal-polluted industrial effluents. Since pH and alkalinity are important factors controlling bioavailability and toxicity of metals to fish, these findings were attributed to the very low pH and alkalinity values of the lake water. Char from SOS were re-examined in September 2010. Sizes of fish caught were similar to those sampled in this lake in the 1990s. A comparison between metal concentration in liver and kidney samples from 1992, 2007 and 2010 show remarkable differences between metal species. While tissue concentrations of Cu and Zn, both elements essential for many metabolic processes such as protein synthesis, remained relatively stable between 1992 and 2010, concentrations of Cd and Pb decreased considerably within this period of time. For instance, Cd and Pb concentrations in the kidney dropped by a factor of 2.0 from approx. 95 mg/kg Cd to 36 mg/kg Cd and by a factor of 5.4 from approx. 25 mg/kg Pb to 4.6 mg/kg Pb, respectively. The decrease of Cd and Pb tissue concentrations coincides with significant changes of lake water conditions over the past two decades. While concentrations of Cd and Pb in the water have dropped by a factor of 100, alkalinity has increased by a factor of 3 and pH increased almost by one unit to pH 6.33. The changes in water conditions could be attributed to climate change driven weathering processes and a shortening in the ice cover duration by several weeks. In consequence, the decrease of Cd and Pb concentrations in char tissues is to be attributed to a decreasing bioavailability of Cd and Pb for fish under more alkaline lake water conditions.

MO216

Biomonitoring potential of five sympatric Tillandsia species for evaluating urban metal pollution (Cd, Hg and Pb) in Asunción (Paraguay)

A. Sánchez-Chardi, Autonomous University of Barcelon / Microscopy Facility Chronic and acute exposure of inhaled pollutants at low concentrations can produce significant toxic effects in biological systems. Atmospheric emissions of metals in urban sites due to diverse anthropogenic activities such as domestic burning of wood or fossil combustibles, waste incineration, traffic or diverse industrial processes are a key scenario of this concern. In addition, in several developing countries, the progressive and unrestrained industrialisation and rapid urbanisation, together with insufficient emission control and shortcomings in environmental legislation, cause serious environmental problems. The present study identifies metal pollution sources in Asunción (Paraguay) using quantification of three non essential heavy metals highly toxic for biological systems (Pb, Hg and Cd) in five autochthonous epiphytic plants from Tillandsia genus (T. recurvata, T. meridionalis, T. durati, T. trichoplepis, T. loliacea) according to different traffic levels (reference, low, medium and high polluted sites). The three metals increased in polluted sites following Pb (till 62.99 ppm in T. trichoplepis) > Cd (till 1.53 ppm in T. recurvata) > Hg (till 0.36 ppm in T. recurvata). Although the species showed similar bioaccumulation pattern (namely, higher levels of metals in polluted sites), enrichment factors reported T. trichoplepis as the most relevant bioindicator due to its wide distribution, abundance, low metal content in control site and high metal contents in polluted sites, and significant correlations with traffic density. This species seems to be a promising biomonitor, which pollution in areas out of air monitoring control such as Asunción, where the high levels of metal pollution especially Pb, may represent an increment of risk for human populations of this urban area.

MO221

A proper Bivalve safety assessment using traditional and original Bioaccumulation Factors

S. Manente, Ca Foscari University of Venice / Department of Molecular Sciences and Nanosystems; A. Zuin, Ca Foscari University of Venice / Molecular Sciences and Nanosystems Department; G. Ravagnan, Ca Foscari University of Venice / Department of Molecular Sciences and Nanosystems

The first comprehensive approach to heavy metal (HM) ecotoxicology in marine environments is to find the bioavailable amount of HM. The aim is normally obtained using BAF (BioAccumulation Factor) determination to total HM into the sediment. Contrary we believe that it would be appropriate to replace BAF with a factor that takes into account the different chemical and physical forms in which HMs are found in the matrix. So, together to the traditional BAF, we also...
considered a factor (BGPh-BAF) that takes into account only bioavailable (B) and potentially bioavailable metal’s geochemical phases (GPh) in the sediment of different areas of Venice Lagoon (Italy), obtained by sequential extraction. Despite the limitations (e.g. the lack of uniformity among operative protocols) HM’s geochemical extraction/fractionation is useful for assessing the relative importance of the different geochemical forms. In particular, we measured 22 HM’s levels both in sediments and in the Mollusca Bivalve Veneridae (intestinal tissues, hepatopancreas and shells), in order to evaluate a true bioaccumulation trend. In fact, since each HM’s speciation form may have different bioavailability and toxicity, it seems rightly concerned about the exact quantification of the different forms of HM present in the aquatic environment. The measurement of total HM may not be able to provide information about the extent of pollution and its relationship on organisms; thus the determination of different fractions, and particularly those bioavailable, assumes great importance to describe real phenomena of bioaccumulation and consequently biomagnifications in trophic chain. In this way, i.e. determining BGPh-BAF, we could obtain numeric (then comparable) values as index both of Bivalve specific uptake paths (metal bioaccessibility) and of pollutants bioavailability in sediments. In some cases, such as for the Cu, it is detected and gives scientific evidence to a diversification in the ability to bioaccumulate some HM present in the BGPh in soft tissues and shells, which is not found in the comparison between the BAF calculated for the total sediment. This result highlights the complex phenomena that govern the processes of bioaccumulation pointing out both organism ability to take on the HM in bioavailable forms and, once the body is exposed, the physiological aspects otherwise undetectable.

**MO218**

**Using a portable XRF analyzer for risk assessment of contaminants in soil at a recreational boatyard**

M. Lagerström, Stockholm University / Institute of Applied Environmental Science ITM; T. Eklund, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES

Recreational boatyards along the Swedish coast have been found to be heavily contaminated as a result of the use of anti-fouling paints on boat hulls. During boat maintenance, spent anti-fouling paints particles end up on the ground resulting in contamination of the soil. Concentrations well above the national guideline values for soil of, amongst others, Cu and Zn are commonly found. However, only 34 out of an estimated total of 2500 boatyards in Sweden have been investigated for soil contamination. During these investigations, an average of only five samples per site were collected, providing limited information on the contamination status of these areas. A handheld X-Ray Fluorescence (XRF) analyzer was tested at two recreational boatyards by lake Mälaren in Sweden to assess its potential in mapping metal contaminants in the surface soil at boatyards. The XRF analyzer is a quick way of measuring metals in soil (2 minutes per measurement) at a considerably lower cost compared to laboratory analysis. Around 50 measurements were carried out in each boatyard through systematic random sampling where the sampling points were randomly distributed in a 1x10m sampling grid. Sieved soil samples (<2mm) were collected for confirmatory laboratory analysis through acid digestion and subsequent analysis on ICP-OES at no less than 10% of the sampling points at each site. The laboratory analyses showed linear correlations to the XRF measurements for Cu, Zn, Pb and Sn. The correlation slopes were close to 1, except for Sn for which the results showed that a correction factor was necessary to apply to the field measurements. Our study demonstrates that the XRF analyzer is a valuable screening tool that can provide a quick and high resolution overview of the contamination situation at a boatyard.

**Emerging contaminants of urban origin: sources, sampling, analytical issues and remediation techniques (P)**

**MO219**

**Analysis of parabens by DLLME-HPLC-DAD during the four season on Mogi river Brazil-SF**

C.A. Galvão, Universidade de Sao Paulo / Chemistry; F.M. Pereira, University of Sao Paulo / USP / engineering of materials; E.M. Vieira, Sao Paulo University / Departamento de Quimica e Fisica Molecular

Parabens are preservatives extensively used to prevent microbial growth in many common care products. Most parabens are frequently found in river water at concentrations reaching from ng L\(^{-1}\) to mg L\(^{-1}\), and their levels depend mainly on the extent of water dilution resulting from rainfall. Discharge of treated wastewater effluent into the river was found to be the main cause of water contamination with parabens. Dispersive liquid-liquid microextraction (DLLME) is an analytical technique, based in the dispersion of extraction solvent assisted with a disperser solvent within an aqueous solution that generates a very high contact area between the aqueous phase and the extraction solvent. The present study reports the application of a DLLME-HPLC-DAD for the simultaneous separation and determination of four paraben preservatives (methyl-, ethyl-, propyl- and n-butyl-paraben) in surface water. The surface water samples were collected during the four seasons of a year period (december 2013, february 2014, may 2014, and august 2014), from Mogi river, on São Paulo state, Brazil. The DLLME-HPLC with diodo array detection method shows a good chromatographic separation for the 4 parabens studied (methyl-, ethyl-, propyl-, and n-buty1). The method exhibit detection limits from 1.0μg L\(^{-1}\) for methyparaben, 1.0μg L\(^{-1}\) for ethylparaben (EP), 0.8μg L\(^{-1}\) prophylparaben (PP), and 1.0μg L\(^{-1}\) butylparaben (BP). Good recovery index for the extraction procedure, from 30.3% (MP) to 68.0% (PP), and good reproducibility (> 95% RSD). Surface water samples collected from Mogi River, showed average levels ranging from: 1.1μg L\(^{-1}\) (EP) to 21.8μg L\(^{-1}\) (MP) during spring season, 1.10μg L\(^{-1}\) (PP) to 27.50μg L\(^{-1}\) (MP) during summer season, 1.64μg L\(^{-1}\) (BP) to 4.80μg L\(^{-1}\) (MP) during autumn season, and 0.88μg L\(^{-1}\) (MP) to 6.76μg L\(^{-1}\) (MP) during winter season. It is believed that these levels are associated with the greatest number of people, residences, and industries located in the river region, which has a higher consumption of products containing preservatives such as food, personal care products, pharmaceuticals hygiene, and others. Acknowledgment: This work was supported by CNPq (150540/2013-2).

**MO220**

**SOLID PHASE EXTRACTION STRATEGIES FOR THE ENRICHMENT AND ISOLATION OF MICROPOLLUTANTS AND THEIR ASSOCIATED POLLUTION PRODUCTS FROM DIFFERENT WATER MATRICES**

A.A. Deeb, University of Duisburg-Essen / Instrumental Analytical Chemistry; T. Schmidt, University of Duisburg-Essen

The occurrence of a large spectrum of pharmaceuticals and personal care products in the environment clearly shows that conventional wastewater treatment plants are not capable of fully removing these compounds. To the detection of micropollutant using ozonation is discussed intensively. A main drawback of ozonation is that it does not lead to a complete mineralization of organic compounds but to the formation of oxidation products (transformation products), which might be potentially toxic. For example, 2,6-dichloroaniline, a toxic and carcinogenic transformation product, has been shown to be formed during ozonation of diclofenac in an aqueous solution. The need for proper sample preparation techniques is a challenging task. Up to date, there is no suitable sample preparation method available for isolation of transformation products produced during wastewater treatment. Sample preparation is a very important and essential step in environmental analysis. The basic concept of sample preparation methods is to convert a real matrix into a sample suitable for analysis. Even the best analytical techniques cannot rectify problems generated by sloppy sample pretreatment. The main goals of sample preparation include (a) removing of potential interferences, (b) increasing the concentration of target analytes in the environment of meaning of enrichment, (c) producing a sample aliquot that will not damage the column or instrument and (d) providing a robust, reproducible method that is independent of variations in the sample matrix. Within this contribution, enhanced solid phase extraction (SPE) procedures that are highly effective for the simultaneous isolation and concentration of acidic, basic and neutral compounds (pharmaceuticals/personal care products and their transformation products following ozonation during wastewater treatment) from different environmental water matrices have been developed. The best configuration for separation of cationic, anionic and neutral (hydrophilic and lipophilic) absorption of samples was developed using a tandem of microporous organic monoliths. Use of ionic monoliths for strong anionic exchanger (Oasis® MAX) in combination with mixed-mode strong cationic exchanger (Oasis® MCX) sorbents without any need for pH adjustment, while mixed-mode strong anionic exchanger sorbent (Oasis® MAX) was used for the isolation of small chain polar acids. High recoveries were obtained in both procedures for all tested compounds. The SPE extraction efficiency of used procedures was examined based on developed and optimized analytical methods.

**MO221**

**Simultaneous analysis of pharmaceuticals residues in water, soil and plants samples using ultrasonic extraction coupled with a solid phase extraction and their determination by HPLC-UV.**

E. Perez-Camero, University of York / Department of Environment; A. Boxall, University of York / Environment Department

An analytical method combining ultrasonic extraction and solid phase extraction (SPE) has been developed to extract and preconcentrate a wide range of nine pharmaceutical compounds including stimulants, antiinflammatories, analgesics, and cardiovascular, hormonal, and neurotransmitter compounds in soil, water, and plants samples followed by their simultaneous determination using liquid chromatography with an UV detector. The extraction method was optimized and evaluated by testing the following variables: extraction solvents, solvent pH and time extraction. Applying 5 and 0.5 g of soil and plant samples and extracting with a mixture of methanol with citric acid (0.1 M) provided the highest recoveries, between 66-90%, with relative standard deviations (RSDs) under 15% for all samples tested. Then, SPE was used to clean-up and preconcentrate the target analytes in the extract using HLB cartridges prior to the analysis through HPLC–UV. The limit of quantifications (LOQs) were between 0.2 and 2 ng g\(^{-1}\) and linearity higher than 0.98 for the majority of the selected pharmaceuticals. The method was successfully applied in several types of samples with different characteristics. The detection of 9 pharmaceuticals including ranitidine, verapamil, tylosin, losartan, diclofenac, ketoprofen, carbamazepine, trimethoprim and simvastatin was achieved at concentration levels of ng g\(^{-1}\).
Chemical analysis and bioassays for the monitoring of wastewater: how many extractions are required for sample preparation? R. Benoit, Chemical Engineering; Z. Baalbaki; A.R. Petosa, McGill University / Chemical Engineering; L. Taylor, McGill University; V.V. Yargeau, McGill University / Chemical Engineering

Contaminants such as pharmaceuticals, herbicides and hormones are of interest due to their unknown or expected impact on the aquatic environment and human health. They are often present in wastewater discharges. Our knowledge on the occurrence of contaminants in the environment is increasing every year but it is still a challenge to extract and analyse them since there is a wide range of compounds and these have different requirements for successful extraction due to their various chemical properties. Moreover, it is becoming increasingly important to consider that these extracts will also be used to prepare samples for diverse bioassays, which allow the analysis of wastewater quality or treatment efficiency not solely based on the removal of selected contaminants but also considering removal of toxicity. To tackle those challenges, various approaches were tried to develop a multi-residue method to extract diverse contaminants including a sweetener, hormones, pesticides, antibiotic and other pharmaceuticals and obtain representative toxicity data. Previous studies have recommended the use of multiple cartridge types and extraction protocols in order to recover a wide range of compounds that would be representative of the sample analyzed. With the objective of limiting cost and time associated with sample preparation for the monitoring of wastewater, we optimized a multi-residue method based on the use of only two different cartridges, OASIS MCB and OASIS MAX. Using this method, we were able to extract acidic, neutral and basic contaminants, and also the analytes were detected in samples of different water types at levels down to pg/L, which is lower than the detection limit for most of the compounds. This method was validated with a set of wastewater samples and performed similarly to a standard solid phase extraction (SPE) method.

MO223 Characterization of iodinated-disinfaction byproducts formed during chlorination and chloramination of different source waters. C. Postigo, IDAEA, CID-CSIC / Institute for Environmental Assessment and Water Research (IDAEA-CSIC); J.J. Ferrer, IDAEA, CID-CSIC / Institute for Environmental Assessment and Water Research

Iodinated DBPs have been recently identified in drinking water. However, most iodinating DBPs still belong to the unknown fraction of the halogenated organic material (~50% of the TOX) formed during water disinfection and hence, remain unidentified [1]. Identified iodinated DBPs have been found to be in some cases more toxic than their chlorine-containing analogues [2]. In this context, the main goal of the present work was to investigate the formation of emerging iodinated DBPs in the production of drinking water from source waters with different iodide and bromide levels. Chlorination and chloramination reactions with two different reference aquatic natural organic matter (NOM) solutions, i.e., Suwanee River NOM and Nordic Reservoir NOM, and with different bromide/iodide content were performed at pH 7.5 and room temperature for 72 hours. Chlorination and chloramination reactions were also carried out with Llobregat River water. Source waters were characterized in terms of their total organic carbon, bromide and iodide concentrations, and SUVA, in order to relate these parameters to disinfection byproduct formation. Reacted waters were extracted onto purified XAD resins for identification and characterization of “unknown” iodinated disinfection byproducts by means of gas chromatography-mass spectrometry (GC-MS) detection. Furthermore, the formation potential of target iodo-trihalomethanes, iodo-haloacetic acids, and iodoacetaldehyde was evaluated. Determination of iodo-containing trihalomethanes and haloacetic acids was performed by means of liquid-liquid extraction (LLE) with methyl tert-butyl ether (MTBE) and subsequent GC-MS analysis. Half of the extract was derivatized with diazomethane for iodo-haloacetic acid analysis. Target GC-MS analysis of iodoacetaldehyde required its derivatization with O-(2,3,4,5,6-pentafluorophenyl)methylhydroxylamine PFBHA and subsequent LLE with hexane. Overall, iodinated disinfection byproducts were formed to a larger extent after chloramination of waters containing high iodide levels. Acknowledgements C. Postigo acknowledges support from the EU 7th Framework Programme (FP7/2007-2013) under grant agreement n° 274379 (Marie Curie IOF). The EU is not liable for any use that may be made of the information contained therein. [1] S.D. Richardson, M.J. Plewa, et al. (2007) Mutat. Res. 636, 178-242 [2] S.D. Richardson, F. Fasano, et al. (2008) Environ. Sci. Technol. 42: 8330-8338

MO224 Optimization of molecularly imprinted solid phase extraction (MISPE) coupled with UHPLC-FD, for the determination of estrogens in wastewaters R. Guedes-Alongo, Universidade de Las Palmas de Gran Canaria / Chemistry; S. Santana-Viera, Universidad de Las Palmas de Gran Canaria / Departamento de Química; S. Montesdeoca-Espadon, Departamento de Química; Z. Sosa-Ferrera, J. Santana-Rodriguez, Universidad de Las Palmas de Gran Canaria / Departamento de Química

Endocrine disrupting compounds (ECDs) are a great group of contaminants, among emerging pollutants which have attracted the attention of the international community due to their capacity of altering the natural hormonal equilibrium, producing harmful effects on human health. Female endocrine disrupting estrogens are considered EDCs because present estrogenic activity. Some authors have linked the concentrations of estrogens in environmental waters with changes observed in aquatic biota as for example changes in reproduction of fish [1]. The levels of EDCs in the environment are usually in the range of ng·L−1. [2]. Therefore, it is necessary to develop new extraction methods that allow the determination of these compounds in complex matrices. Molecularly Imprinted Solid Phase Extraction (MISPE) is based in the use of a molecularly imprinted polymer as stationary phase of solid phase extraction which allows a selective extraction of a kind of compounds from a matrix. In this study, a molecularly imprinted solid phase extraction (MISPE) coupled to ultra-high performance liquid chromatography with fluorescence detection has been optimized to determine four estrogens (estrone, 17β-estradiol, 17α-ethinylestradiol and 17β-estradiol-3-methyl ether). Some parameters of the extraction process as pH of the sample and elution volume and the separation and detection process have been optimized. The method developed showed good recoveries (over 80%) and reproducibilities for the four estrogens in samples of wastewater treatment plants from Gran Canaria (Spain) which use different water treatment processes. Moreover, this method was successfully applied to environmental uncontaminated water samples, which were collected in different areas of the island. The method was validated with a set of wastewater samples and performed similarly to a standard solid phase extraction (SPE) method.

MO225 Molecularly imprinted polymer-based solid phase microextraction fibers for the selective separation and enrichment of the antiviral drug Abacavir from aqueous matrices Z. Terzopoulou, M. Papageorgiou, G. Kyzas, D. Bikias, D. Lambropoulos, Aristotle University of Thessaloniki / Chemistry

The environmental release of antiviral drugs, like other active pharmaceutical ingredients, is of considerable concern due to potential ecosystem alterations and the development of viral resistances. Based on these concerns and the potential risk for occurrence of antiviral drugs deriving from wastewater, there is need for sensitive and reliable separation and enrichment methods. Solid phase microextraction (SPME) is currently one of the most popular pre-treatment techniques for extracting and enriching analyte from environmental samples and thus development of new SPME sorbents has received considerable attention. Recently, the removal and enrichment of pharmaceuticals with selective adsorption of target compounds from water using molecularly imprinted polymers (MIPs) is considered as one of the most promising techniques, since MIPs have specific binding sites with complementary size, shape, and functional groups to the template molecule and thus can recognize targets with high selectivity. In this light, novel MIP-based SPME fibers for the selective separation and enrichment of antiviral drugs from aqueous matrices were prepared. Abacavir, which is a HIV-1 reverse transcriptase inhibitor, was chosen as the model compound. Two water-soluble MIPs were prepared using FT-IR spectroscopy, SEM and X-ray Diffraction. The performance of the prepared SPME MIPs was evaluated by various parameters (i.e. pH, contact time, temperature, initial compound concentration etc.). The developed method by using LC-MS was thoroughly validated for its linearity, selectivity, precision and accuracy. Under the working extraction conditions, the proposed method showed good linearity in the range of 50-1000ng/mL, repeatability of the extractions (RSD ≤ 4.3%, n=3), and low limits of detection (≤ 5ng/L). This method combined the advantages of MIPs and SPME, and it could become an alternative tool for analyzing the residues of antiviral drugs in complex water matrices, such as wastewaters. Acknowledgements The support for this study was received from the Greek Ministry of Education and Religious Affairs through Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF)-Research Funding Program “Excellence II (Aristea II)” under the title “Advanced microextraction approaches based on novel nano-polymerization technologies to measure pharmaceuticals, personal care products and their transformation products in the aquatic environment”, which is gratefully appreciated.

MO226 Preparation of molecularly imprinted polymers for MISPE extraction endocrine disruptors, drug abuse and drugs in wastewater samples of the Greater ABC region, SP, Brazil V.d. Bianchi, UFABC / CCNH; A.N. Soares, UFABC Universidade Federal do Grande ABC / Centro de Ciências naturais e Humanas; E. de Lima, Universidade Federal do ABC / Centro de Ciências naturais e Humanas

The sample preparation prior to instrumental analysis is an indispensable prerequisite for the establishment of a chromatographic and / or electrophoretic sensitive and selective method for the trace analysis in complex matrices. New
trends in sample preparation for clinical and pharmaceutical analysis address the molecular recognition as the extraction based on immunoaffinity and molecular imprinting polymers (MIPs). From these materials it is possible to remove the analyte of interest in the sample eliminating most interfering compounds such as proteins, salts, acids, bases and other organic compounds with properties similar to analytes. The molecular imprinting materials are stable for long-term storage, are easy to prepare and economical. In our group we use the reaction processes for bulk and aqueous solution for the determination of estrogen hormones, α-THC, cocaine, MDMA in effluents of the Greater ABC region which is in the metropolitan area of São Paulo. The results show that the polymers obtained through bulk pores have a size < 20µm and more uniform than the others obtained through precipitation. Factors that improve the retention of analytes of interest in these materials. The authors thank FAPESP Process 2013 / 12569-8 by the aid granted to carry out this work.

MO227
An effective, easy and cheap way of monitoring widely used antibiotics in a catchment area
E. Tzelepi, Lancaster University / Centre for Global EcoInnovation; C.J. Halsall, Lancaster University / Lancaster Environment Centre; K. Waterhouse, Bowland Analytical Support Ltd.
Over the last decade the potential impacts of pharmaceuticals in the environment have attracted increasing interest from the scientific community and the media. The main issue is that the occurrence of pharmaceuticals is closely related to the emergence and development of resistance to antibiotics. Therefore, it is critical to develop better methods for monitoring these contaminants and for the labelling of these drugs to improve patient treatment and reduce indiscriminate use. This study aims to establish a mass flow diagram and determine the percentage of antimony and trioxide hazardous from traditional landfilling to recycling and incineration approaches that aim at materials and energy re-use. In addition to materials and energy, waste also contains a large variety of emerging contaminants, and yet how landfilling, incineration, and recycling can affect environmental emissions and how to evaluate the risks and benefits of such emerging contaminants remains largely overlooked. Antimony is an up and coming emerging contaminant, as it is increasingly used in diverse products as a flame retardant that can replace brominated flame retardants (BFRs). The effects of antimony in the environment, remain largely unknown. WASTEFFECT is a research project funded by the Norwegian Research Council where the main goal is to develop robust waste emission and exposure models for waste regulators and companies to anticipate and reduce risks from emerging contaminants. Therefore, one of the main part of this project is to determine how emerging contaminants behave in different waste streams, like landfilling, incineration and recycling. For priority contaminants used in consumer products, waste treatment will play a large role in the ultimate removal of these substances from the environment. As case studies of emerging contaminants, we have established a Norwegian waste emission inventory of legacy and new brominated flame retardants (BFRs), bisphenol A and antimony, in EE-waste, car waste, plastics, glass and combustible waste. Input parameters for these inventories have been obtained by sampling and analysing air, leachate and solid samples from various waste treatment methods, including incineration, recycling and landfilling, by considering the entire waste-life cycle. Antimony results will be used to establish a mass flow diagram and determine the percentage of antimony recycled, removed or emitted in to the Norwegian environment through waste handling like recycling, thermal treatment or landfilling. The data will be presented in the poster. These data (along the ones obtained for BFRs and bisphenol A) will be used to estimate the contribution of the waste sector to the net burden or excessive to the environment and humans. The main output of this work will help guide the waste management sector on which of the treatment methods provide the lowest risk in regards to emerging chemicals, and which processes can be optimized.

MO230
WASTEFFECT: Life cycle effects of emerging contaminants in waste with focus on antimony (Sb)
H.H. Arp, NGI / Environmental Engineering; G. Okkenhaug, Norwegian Geotechnical Institute; A.R. Almas, Norwegian University of Life Science; S.E. Hale, Norwegian Geotechnical Institute / Environmental Engineering; N.A. Morin, Environmental Engineering; M.M. Sparrevik; P.L. Andersson, Umea University / Chemistry Department; K. Breivik, Norwegian Inst. for Air Research; F. Wanja, University of Toronto at Scarborough / Department of Physical and Environmental Sciences; G.D. Breedveld, Norwegian Geotechnical Institute / Department of Environmental Engineering
Waste treatment has become a dynamic sector, which in many countries is changing from traditional landfilling to recycling and incineration approaches that aim at material and energy re-capture. In addition to materials and energy, waste also contains a large variety of emerging contaminants, and yet how landfilling, incineration, and recycling can affect environmental emissions and how to evaluate the risks and benefits of such emerging contaminants remains largely overlooked. Antimony is an up and coming emerging contaminant, as it is increasingly used in diverse products as a flame retardant that can replace brominated flame retardants (BFRs). The effects of antimony in the environment, remain largely unknown. WASTEFFECT is a research project funded by the Norwegian Research Council where the main goal is to develop robust waste emission and exposure models for waste regulators and companies to anticipate and reduce risks from emerging contaminants. Therefore, one of the main part of this project is to determine how emerging contaminants behave in different waste streams, like landfilling, incineration and recycling. For priority contaminants used in consumer products, waste treatment will play a large role in the ultimate removal of these substances from the environment. As case studies of emerging contaminants, we have established a Norwegian waste emission inventory of legacy and new brominated flame retardants (BFRs), bisphenol A and antimony, in EE-waste, car waste, plastics, glass and combustible waste. Input parameters for these inventories have been obtained by sampling and analysing air, leachate and solid samples from various waste treatment methods, including incineration, recycling and landfilling, by considering the entire waste-life cycle. Antimony results will be used to establish a mass flow diagram and determine the percentage of antimony recycled, removed or emitted in to the Norwegian environment through waste handling like recycling, thermal treatment or landfilling. The data will be presented in the poster. These data (along the ones obtained for BFRs and bisphenol A) will be used to estimate the contribution of the waste sector to the net burden or excessive to the environment and humans. The main output of this work will help guide the waste management sector on which of the treatment methods provide the lowest risk in regards to emerging chemicals, and which processes can be optimized.
information of the actual situation of biocide emission into the environment. As regulatory authority we are interested if the consequences of the BPR are already observable (e.g. practicability of risk mitigation measures, exclusion and substitution of substances with very high concern). To improve our knowledge about the impact of biocides to the environment it was the aim of this case study to investigate the biocides cybutryne, propiconazole and tebuconazole retrospectively by analysing suspended particulate matter (SPM) samples of the German Environment Agency (UBA). Cybutryne is used as construction material preservative for façades and insulating material until it was banned for this use in 2011. An increase in use of other material preservative substances (e.g. tebuconazole, propiconazole) is therefore expected. Both active substances are probably endocrine disruptors and are persistent in the environment. The potential release of cybutryne, tebuconazole and propiconazole from material preservatives into surface water was examined in SPM samples from the years 2006 to 2012 from six different sampling areas (e.g. at the rivers Rhine and Elbe). All areas are assumed to be dominated by storm water and municipal waste water whereas the agricultural influence was rather secondarily. The results show that concentrations of all three substances are similar at nearly all locations. For cybutryne decreasing concentrations were detected at two the sampling site. Comparable decreases were observed for propiconazole and tebuconazole at one sampling site of the river Rhine. But in most cases no significant trends are observed over time. We conclude that further investigations over a longer period are necessary to observe the effect of exclusion and substitution of substances. This study is part of a UBA research project with the question “Validation of a proposed or biocides and development of a monitoring program for biocides in Germany”.

MO234
Partitioning of biocides between water and inorganic phases of render
M. Urbanecvik, Aarhus University Department of Environmental Science; U. Bolsmann, Aarhus University; B. Bester, Aarhus University / Environmental Science
The use of biocides as additives for building materials has gained importance in recent years. These biocides are, e.g., applied to renders and paints to prevent them from microbial spoilage. However, these biocides can leach out into the environment. In order to better understand this leaching, the partitioning of biocides between water and inorganic phases of render was studied. In this study the partitioning constants of benzisothiazolinone, carbendazim, dichloroaceticisothiazolinone, diuron, iodocarbox, isoprotron, irgarol, mecoprop, methylisothiazolinone, octylisothiazolinone, terbutryn, and tebuconazole towards minerals were studied. A mixture of biocides listed above was used to estimate partitioning constants between water and five different minerals, commonly used as fillers in renders: barite, calcium carbonate, kaolinite, mica and talc. The resulting K values for all minerals analysed were compared. The partitioning constants for calcium carbonate varied between 0.1 (isoprotron) and 1.1 (iodocarb) and 84.6 (dichloroaceticisothiazolinone), respectively. The results for barite, kaolinite and mica were in a similar range and usually the compounds with high partitioning constants possessed the highest values for one mineral. From all minerals investigated, only talc showed high partitioning for all studied biocides. Partitioning constants for talc vary from 2.2 (benzisothiazolinone), 778.1 (carbendazim) and 435.6 (dichloroaceticisothiazolinone), respectively. The obtained partitioning constants were compared with render-water distribution constants, which showed that leaching of biocides, might be estimated based on partitioning constants for individual components of the render.

MO235
An integrated experimental and computational approach for characterizing the kinetics and mechanism of triadimefon racemization
I.K. Kempek, National Exposure Research Laboratory; Q. Cheng, Korea Institute for Advanced Study / School of Computational Science; Q. Teng, US EPA / National Exposure Research Lab; S.A. Marchitti, U.S. EPA / National Exposure Research Laboratory
Enantiomers of chiral molecules commonly exhibit different environmental fates, pharmakoecinetics, and toxicities. Ignoring these differences can introduce significant uncertainty when modeling the physical and environmental fate of chiral chemicals and evaluating their risk to human health and the environment. The stereoselective transformation of chiral compounds under biological and environmental conditions has been well studied, however, there is far less information on the biological and environmental racemization (and enantiomerization) of chiral compounds. Racemization is the macroscopic process of the irreversible, reversible conversion of one enantiomer into the other. From all the racemic mixture, while enantiomerization refers to the microscopic and molecular process of the reversible conversion of one enantiomer into the other. These processes have been shown to occur with environmental chemicals and pharmaceuticals and can have a profound impact on efficacy, toxicity, and sample preparation, storage, and analysis, particularly when they occur unknowingly. Triadimefon [(RS)-1-(4-chlorophenyl)-3,3-dimethyl-1-(1H,1,2-triazol-1-yl)butan-2-one] is a systemic broad spectrum 1,2,4-triazole fungicide that has been shown to undergo racemization in soils, water, and organic solvents. We utilized a series of NMR spectroscopic and GCMS techniques to measure the rates of enantiomerization and racemization, deuterium isotope effect, and activation energies for triadimefon in H2O and D2O. From these results we were able to determine that the alpha-carbon of triadimefon is the reaction site, cleavage of the CH(CD) bond is the rate determining step, the reaction is base-catalyzed, and the reaction likely involves a symmetrical intermediate. Computationally, we applied the B3LYP/6-311+G** level of theory to compute optimized geometries, harmonic vibrational frequencies, natural population analysis, and intrinsic reaction coordinates for triadimefon in water and were able to hypothesize three racemization pathways. The experimental and computational results including the activation energies and the Gibbs free energies of the reaction agreed well with each other. This work provides an initial step in developing predictive, structure-based models that are needed to quickly identify both pharmaceutical and non-pharmaceutical compounds that may under undergo racemization.

MO236
Degradation of cytostatic drugs: hydrolysis, solar box, UV, UV-H2O2 and bioreactor
H. Franquet, Environmental Chemistry; A. Medina, C. Sans, Universitat de Barcelona / The Lundin of Chemical Engineering; S. Lacorte, IDAEA-CSIC / Environmental Chemistry
Cytostatic drugs, used in chemotherapy, are partially excreted unchanged by urine and faeces. These compounds are directed to wastewater treatment plants (WWTPs), where some can be degraded while others are recalcitrant and
discharged to receiving waters by the WWTP effluents. Some authors studied their behaviour under different degradation processes [1-4], although degradation kinetics and pathways need further attention to determine their fate in the environment. The aim of this work was to evaluate the degradability of five treatments, namely hydrolysis, Solarbox, UV, UV-H2O2 and biodegradation. To perform the experiments, sixteen cytostatic drugs were selected; those affecting the DNA, those metabolized in the liver and those those degraded under UV light (melphalan, etoposide) and some others were found to be stable using biodegradation or UV, making necessary the use of advanced oxidation processes (AOPs) such as UV-H2O2 to eliminate them (ifosfamide, cyclophosphamide). Degradation constants were calculated in order to define the stability of this new family of contaminants under laboratory conditions. [1] Buergi U, Buser HR, Poiger T and Müller MD. 2006. Occurrence and fate of the cytostatic drugs cyclophosphamide and ifosfamide in wastewater and surface waters. Environ. Sci. Technol. 40: 7242-7250 [2] Kosjek T and Heath E. 2011. Occurrence, fate and determination of cytostatic pharmaceuticals in the environment. TrAC, Trends Anal. Chem. 30: 1065-1078 [3] Negreira N, López de Alda M and Barceló D. 2014. Study of the stability of 26 cytostatic drugs and metabolites in wastewater under different conditions. Talanta 121: 385-398 [4] Li, Chang VW, Giani A and Wang YJ. 2013. Removal of cytostatic drugs from aquatic environment: A review. Sci. Total Environ. 445-446: 281-298

MO237 Photodecomposition of sulfamethoxazole under simulated sunlight irradiation: transformation products and residual antibacterial activity toward Vibrio Fischeri

M. Gmuruk, Lodz University of Technology / Faculty of Process and Environmental Engineering Department of Bioprocess Engineering; M. Majewsky, Karlsruhe Institute of Technology (KIT) / Engler Bunte Institute Chair of Water Chemistry and Water Technology; H. Horn, Karlsruhe Institute of Technology KIT / Chair of Water Chemistry and Water Technology

Sulfamethoxazole (SMX) is a bacteriostatic antibiotic which is only partially metabolized during therapy. As a result, it reaches the aquatic environment in its unchanged biologically active form. Conventional wastewater treatment is not effective in removing SMX. Consequently, it has been detected in urban wastewater and surface waters due to the inability of environmental bacteria to transmit and acquire antibiotic resistance, the latter has become a global concern. Therefore, it is crucial to better understand photochemical processes occurring in natural water just as the formation of active transformation products (TPs). The main goal of the presented study was to investigate the TPs formed during SMX photolysis and to determine their antibacterial toxicity in mixture. To simulate the natural conditions under sunlight irradiation, a UHPLC/QToF hybrid mass spectrometer was used for the determination of accurate masses. Vibrio Fischeri growth inhibition (GI; 24h) and luminescence inhibition (L; 30 min; 24h) were used to determine the changes of antibiotic activity in mixture during simulated sunlight irradiation. During long-term SMX photolysis experiments lasting for 3 weeks (Cas 10/100mg), nine TPs could be identified by reference standards. Moreover, three further TPs of photodecomposition of SMX were found. Some of the TPs (e.g. N-acetyl-SMX) were observed to be formed during photolysis for the first time. Furthermore, a TP with m/z 270 was tentatively confirmed as dihydroxylated SMX. The kinetics of the TPs were determined and correlations between TPs were established. The DOC mass balance clearly showed that only around 5 to 10% were mineralized during the experiment emphasizing the need to elucidate the fate of TPs. The toxicity analysis confirmed that the mixture retains its toxicity toward luminescence (30min; 24h) and that there is no change over the treatment time on EC50. In contrast, growth inhibition activity was found to decrease over the irradiation time, however, this decrease was not proportional to the transformation of the parent compound SMX.

MO238 Householder contribution to glyphosate losses in urban storm drains

C. Ramwell, M. Kah, University of Vienna / Department of Environmental Geosciences; P. Johnson, Alabama Department of Conservation and Natural Resources / Alabama Environmental Institute

Glyphosate has been detected at low levels in some water bodies and this is increasing interest in the contribution of urban areas. Glyphosate is widely used by both the amenity sector and by householders. In order to focus mitigation measures appropriately, it is necessary to ascertain the extent to which different sources contribute to any pollution. The extent to which glyphosate use in the home and garden sector may contribute to surface water contamination has not previously been quantified. The aim of this study was to quantify the widely-used herbicide, glyphosate, and its degradation product, aminomethylphosphonic acid (AMPA) in surface water drain (storm drains) that could be attributed to householder usage alone. Water samples were taken from a residential area in York, England where all the surface water from the catchment drained to a single point. Samples were taken in June/July during one of the main application periods. Maximum glyphosate and AMPA concentrations in surface water drains were 8.99 and 1.15 µg/L respectively after the first rain event period, but concentrations rapidly declined to < 1.5 µg/L glyphosate and < 0.5 µg/L AMPA. The AMPA:glyphosate ratio was typically 0.35. Less than 1% of the applied glyphosate was recovered in drain water. Despite over-dosing occurring, glyphosate concentrations in drain flow were lower than concentrations reported elsewhere from professional use in urban areas.

MO239 The Inventory, Environmental Releases and Risk Assessment for Short-Chain PFASs in China

X. Yu, H. Peking University; Y. Cao, J. Wang, J. Liu, Peking University

Since 3M Company voluntarily phased out of PFOS during 2000-2002, risk management of Per- and Polyfluorooalkyl substances (PFASs), represented by PFOS and PFOA as 8C PFASs, has already been one of the leading works in global chemicals management. For long-chain PFASs, due to the significant environmental and health risks caused by the specific persistent, bioaccumulative and toxic characteristics, international communities have successively conducted risk assessment and management focusing on 8C-based long-chain PFASs, and devoted to developing short-chain PFASs as alternatives. Since being used on a global scale, concentrations of short-chain PFASs in the aquatic environment increased year by year and researches pointed out that short-chain PFASs might become the major PFASs in drinking water in the future. High concentrations of short-chain PFASs may endanger the environment and health risks caused by the specific persistent, bioaccumulative and toxic characteristics, international communities have successively conducted risk assessment and management focusing on 8C-based long-chain PFASs, and devoted to developing short-chain PFASs as alternatives. Therefore, it is crucial to better understand photochemical processes occurring in natural water just as the formation of active transformation products (TPs). The main goal of the presented study was to investigate the TPs formed during SMX photolysis and to determine their antibacterial toxicity in mixture. To simulate the natural conditions under sunlight irradiation, a UHPLC/QToF hybrid mass spectrometer was used for the determination of accurate masses. Vibrio Fischeri growth inhibition (GI; 24h) and luminescence inhibition (L; 30 min; 24h) were used to determine the changes of antibiotic activity in mixture during simulated sunlight irradiation. During long-term SMX photolysis experiments lasting for 3 weeks (Cas 10/100mg), nine TPs could be identified by reference standards. Moreover, three further TPs of photodecomposition of SMX were found. Some of the TPs (e.g. N-acetyl-SMX) were observed to be formed during photolysis for the first time. Furthermore, a TP with m/z 270 was tentatively confirmed as dihydroxylated SMX. The kinetics of the TPs were determined and correlations between TPs were established. The DOC mass balance clearly showed that only around 5 to 10% were mineralized during the experiment emphasizing the need to elucidate the fate of TPs. The toxicity analysis confirmed that the mixture retains its toxicity toward luminescence (30min; 24h) and that there is no change over the treatment time on EC50. In contrast, growth inhibition activity was found to decrease over the irradiation time, however, this decrease was not proportional to the transformation of the parent compound SMX.

MO240 PERFLUOROALKYL SUBSTANCES (PFASs) IN TEXTILES

D. A. Micheluzza, Eurofins GfA GmbH; F. Neugebauer, Eurofins GfA Lab Service GmbH / RD. S. Selke, Eurofins GfA Lab Service GmbH; U. kaleix; BUND; M. Santen. In recent years, perfluorinated compounds (PFCs), particularly perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) have been described as compounds of increasing environmental concern and numerous studies were conducted to investigate the environmental distribution and fate of these compounds. As a result, inventories of sources was established based on survey and analysis of the production facilities, processes and use information for PFASs. Besides, emission amounts and factors were estimated to calculate environmental releases of short-chain PFASs. Furthermore, preliminary assessment of environmental risk was conducted. Although the inventory and environmental releases may be coupled with uncertainties, this study provides sufficient information for follow-up scientific researches and risk management of short-chain PFASs. Keywords: PFASs, inventory, environmental releases, risk assessment

MO241 Exposure and risk in indoor environment: VOCs analysis from unexpected microbiological sources

A. Micheluzza, Ca Foscari University of Venice / Molecular Sciences and Nanosystems; M. Rovea, Agenzia Reg Prevenz  Protez Ambientale Veneto AR / Dipartimento Prov Padova; G. Formenton, ARPAV; S.-V. Manente, Ca Foscari University of Venice / Department of Molecular Sciences and Nanosystems; V. Prigione, University of Turin; V. Tigini, University of Turin; F. Pinzari, Consiglio per la Ricerca e la sperimentazione in Agricoltura/Agricultural Research Council; G. Vares, University of Turin / Dept of Life sciences and systems biology; G. Ravagnan, Ca Foscari University of Venice

In recent years, studies were focused on volatile organic compounds (VOCs) originating from biological sources and their relation to the air quality in indoor environments, like archives, libraries, museums, schools, offices, etc. In these climate controlled environments, particular fungal species are able grow, producing several volatile organic compounds that are suspended in the air or adsorbed on dust particles. The assessment of their nature is needed for a proper analysis of the indoor air quality, in order to understand the potential risk for the human health of workers and visitors. To propose fast remediation to avoid “sick building syndrome” phenomena, a rapid tool to understand fungal contamination in indoor environments could be air sampling followed by gas chromatography-mass spectrometry (GC-MS) analysis. For the broad speciation of unknown trace of VOCs we tested evacuated stainless steel canisters to sample the indoor air within a few seconds. The composition of the indoor air in a Ca Foscari
University Library, furnished with the innovative closed metal cabinets called Compacstore® and affected by an active mold infection was analyzed with the aim of detecting a specific chemical fingerprints of fungi. Six canisters were adopted in different areas of the deposit to collect VOCs and subsequently analyzed by GC-MS. Moreover, laboratory experiments were developed to collect VOC productions directly from the dominant fungal species isolated from the library by previous sampling and identified by morphological and molecular methods of analysis (Euromonitor, E. chevalieri, Penicillium pinophilum, A. creber, Penicillium chrysogenum, P. brevicaespitem pulcherrimum, and Chondosporium cladosporoides). All the samples were monitored for 1 month by weekly analysis of the emitted VOCs. For all the analysis, microscale purge & trap Techt 7100 was adopted as sampling and pre-concentration system directly connected with corresponding sampling devices (cartridges and culture bottles) and GC-MS. Several volatile organic compounds, both known (i.e. 1,4-pentadiene and 2-butanone) and unknown emerging substances, that were detected by the direct analysis of indoor air were also found in the emissions of the dominant fungal species isolated from the library by microbiological and chemical analysis. The results suggest a close relationship between the fungal infections and the poor indoor air quality.

MO242
Polychlorinated biphenyls and flame retardants in indoor and outdoor air: evaluation of seasonal variability and relationship with house characteristics

P. Bohlin Nizzetto, NILU Norwegian Institute for Air Research; L. Melymuk, P. Kukucka, Masaryk University, Faculty of Science; RECETOX / RECETOX Research Centre for Toxic Compounds in the Environment; P. Cupr, Masaryk University, Faculty of Science; RECETOX / RECETOX Research Centre for Toxic compounds in the environment; J. Klanova, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; X. Fang, University of York / Dept of Computer Engineering

Indoor air is affected by a wide range of pollutants arising from sources both inside and outside buildings. This study aimed to evaluate indoor air concentrations of semivolatile organic contaminants (SVOCs) across a range of residential environments with respect to house characteristics, indoor materials, and the ratios with outdoor concentrations. The targeted compounds were 7 polychlorinated biphenyls (PCBs), 4 polybrominated diphenyl ethers (PBDEs) and 12 novel halogenated flame retardants (NFRs). Air sampling was conducted in 17 homes in residential areas of Brno, Czech Republic using polyurethane foam passive air samplers (PUF-PAS). PUF-PAS were deployed concurrently inside and outside each home for 28 days, once during summer and once during winter. General sampling rates of 1.4 and 3.5 m$^3$/day were used to obtain air concentrations indoors and outdoors, respectively. The selected houses had various characteristics (i.e. year of construction, renovation, type of house) and house contents (e.g. floor type, furniture, electronics etc.). The range of indoor air concentrations of OPBCs were 39-174 pg/m$^3$ (mean 78.8 pg/m$^3$) in summer and 6.2-86 pg/m$^3$ (mean 52 pg/m$^3$) in winter. Outdoor concentrations of PCBs were 17-65 pg/m$^3$ (mean 27 pg/m$^3$) and 5.3-18 pg/m$^3$ (mean 12 pg/m$^3$) in summer and winter, respectively. Indoor to outdoor ratios (I/O) for PCBs ranged from 1.2-6.4 (mean 3.1) in summer and 0.4-11 (mean 5.1) in winter. Indoor air concentrations of IPBDE were 2.0-65 pg/m$^3$ (mean 14 pg/m$^3$) in summer and 0.5-78 pg/m$^3$ (mean 13 pg/m$^3$) in winter. IPBDEs outdoors were 3.5-66 pg/m$^3$ (mean 12 pg/m$^3$) and 1.1-6.2 pg/m$^3$ (mean 3.0 pg/m$^3$) in summer and winter, respectively. The I/O ratios of IPBDEs were 0.4-5.0 (mean 1.4) in summer, and 0.3-20 (mean 4.7) in winter. INFR indoor air concentrations were 15.22 pg/m$^3$ (mean 222 pg/m$^3$) in summer and 11-776 pg/m$^3$ (mean 102 pg/m$^3$) in winter. INFR outdoors were 2.5-2340 pg/m$^3$ (mean 153 pg/m$^3$) and 0.13-26 pg/m$^3$ (mean 3.7 pg/m$^3$) in the summer and winter, respectively. For INFR, the summer I/O ratios were 0.7-33 (mean 5.6) while in winter I/O ratios were 3.2-1100 (mean 140). The indoor concentrations were significantly higher (<p <0.05) than outdoor concentrations for PCBs in summer and winter, IPBDEs in winter, and INFR in winter. Higher concentrations indoors than outdoors were also found for PBDEs and NFRs in summer but differences were not significant.

MO243
Emerging flame retardants in indoor environment: a multi-site location study conducted in the UK

K. Kademoselou, University of Reading / geography and Environmental Sciences; C.D. Collins, Reading University / Soil Research Centre

Flame retardants (FRs) are man-made chemical compounds widely used in industry during the manufacturing of various commercial products such as computers, plastics, fabrics, textiles and polyurethane foam products in order to minimise or prevent fire and smoke hazards. FRs are used in the production of polybrominated diphenylethers (PBDEs) have resulted in the production of new PBDE-replacement products, also known as emerging FRs (Stapleton et al., 2008). Sampling sites representing three different indoor environments (houses, stores and libraries) were selected at the area of Reading (UK) and the area of Oslo (Norway) respectively. N=18 samples of indoor dust were collected from vacuum cleaner bags in houses, stores, offices and libraries in the area of Reading (UK) during August - December 2013. N=10 samples of indoor dust were collected during November 2013-April 2014 from vacuum cleaner bags in houses from the wider area of Oslo (Norway) as a part of a cohort study of N=60 people within the framework of the ‘Advanced Tools for Exposure Assessment and Biomonitoring’ (A-TEAM) project, a Marie Curie Initial Training Network aiming to establish tools for human exposure biomonitoring of emerging FRs. In the present study the emerging FRs examined are: 1,2- bis(2,4,6-trimorpholxyloxyethane) (BTBPE), 2-ethylhexyl-2,3,4,5-tetramobenzobenzoate (EH-TBB) and bis(2-ethylhexyl)-3,4,5,6-tetramobenzophenolate (BEH-TEBP). Prior to extraction, all samples were spiked with 13C-labelled TBB, TBPH and BTBPE used as internal standards. BDE-77 and BDE-128 were used as recovery standards for EH-TBB, BTBPE and BEH-TEBP respectively. A two-step Solid-phase extraction (SPE) using Florisil (step 1) and 44% acidified silica gel (step 2) was performed coupled with ultrasonication-assisted solvent extraction. The instrument analyses were conducted using a Thermo Scientific ITQ 1100 GC- Ion Trap MS on Electron Ionisation (EI) mode. We report levels of emerging FRs in British and Norwegian indoor environments where humans spend eight hours minimum on a daily basis. Preliminary results will be presented on how variability from diverse indoor dust sources such as houses, stores and offices can affect the levels of emerging FRs, as well as to compare the geographical trends of emerging FRs between Norway and the UK. This project is financially supported by the European Commission FP7 Marie Curie Initial Training Network “A-TEAM” grant number 316665.

MO244
Rapid screening for oxidative characteristics of fine particle collected from different regions in South Korea

J. Kim, School of Environmental Science and Engineering; T. Jeong, Gwangju Institute of Science and Technology; J. Park, S. Kim, School of Environmental Science and Engineering

Many studies have demonstrated the association between particulate matter (PM) and adverse effects on human health including increased respiratory disease and lung cancer. It is well understood that PM2.5 (diameter < 2.5um) can reach the alveolar wall and generate oxidative stress. Oxidative stress is an important mechanism to the PM-induced health effect. Dichlorofluorescin (DCFH), glutathione (GSH), and dithiothreitol (DTT) assay are commonly used for assessing the oxidative stress of PM. Particularly, DTT assay is a cell-free assay, and can rapidly and quantitatively measure the oxygen capacity of PM. PM is heterogeneous, and its properties vary remarkably according to source areas. Therefore, the toxicity investigation of PM at the different regions is necessary. In this study, we collected the PM from various source areas (heavy traffic, industrial, urban, suburban etc.) and assessed its oxidative characteristics using the modified DTT assay and biological assay tools. DCFH and GSH for reactive oxygen species generation were measured to observe the cellular effect of PM using human alveolar epithelial cell (A549). The oxidative characteristics of PM showed the significant difference by sampling site. This result might be attributed to the various chemical composition of PM. Furthermore, a noteworthy relationship was observed between cellular ROS and modified DTT result. This study suggests that 1) the chemical composition of PM highly contribute to its toxicity, and 2) the modified DTT assay has the possibility to apply for measuring the quantitative oxidative effect of PM as good alternative assay.

MO245
Organo phosphorus flame retardants (OPFRs) in outdoor urban atmospheric particulate matter

D. Ramos García, CSIC; S. Lacorte, IDAEA-CSIC / Environmental Chemistry; B.L. van Drooge, University of York / Chemistry

This work presents an analytical procedure for the quantification of organophosphorus flame retardants (OPFRs) in atmospheric particulate matter (PM). The analytical method is based on Soxhlet extraction and a clean-up step with solid phase extraction (SPE). Detection and quantification was performed by gas chromatography-electron impact mass/mass (GC-EL-MS/MS). The validation included blank tests in order to see the background levels of these compounds and percentage of recoveries with spiked filters at two different levels. Ambient air PM samples were collected at an urban background site in Barcelona under contrasting meteorological conditions in order to study the influence of particle loads and meteorological conditions on the abundance of OPFRs. The presence of OPFRs has been determined and substantial correlations have been observed between the levels of certain OPFRs and particle loading of the atmosphere. The presence of individual compounds in ng/m$^3$ concentrations levels in the fine particle mode (PM1) at an urban background site, suggests a potential risk of these compounds to human health.

MO246
Exploiting new technologies to monitor pollution in cities

A. Bonallack, University of York / Environment Department; E.E. Burns, University of York / Chemistry; E. Koutsoumpeli, University of York / Electronics; G. Makraí, University of York / Dept of Computer Science; K.E. Stevens, University of York / Environment Department; X. Fang, University of York / Dept of Computer Science; X. Gao, University of York / Environment Department; M. Kruza, York University / Environment; F. Medhat, M. Parmar, University of York / Electronics; R. Sivengwa, University of York / Department of Sociology; C. Wang, University of York / Chemistry; M. Deplede, European Centre for Environment and Human Health ECHEHH; D.W. Kolpin, U.S. Geological Survey; F. Pillia, Trinity College Dublin / Department of Civil Structural and Environmental Engineering

With an increasing world population and subsequent urbanisation, the need to
address the resultant amplification of public health and environmental issues has become crucial, requiring cost-effective approaches to pollution monitoring, data management, and interpretation. Considering the increased complexity of urban pollution and the lack of monitoring schemes for emerging contaminants, it is imperative to enhance and update the currently used methods and employ faster and more efficient approaches for monitoring pollutants. In this paper, we present the conclusions from an expert workshop that aimed to develop a framework for understanding the exposure of city environments and citizens in the urban area. The poster will illustrate how state-of-the-art analytical techniques can be combined with innovative sampling technologies, such as citizen science, robotics and specimen banking to monitor air, water and soil within city environment. The poster will discuss how these methodologies meet the needs of end users and stakeholders, and how they could offer prompt and robust decision-making support. We recommend the development of a ‘smart city’ model for monitoring urban pollution which can potentially be tailored and adjusted to suit the needs and characteristics of cities of varying size and nature.

MO247
Development and validation of a multi-residue analytical method for the simultaneous determination of antibiotics for monitoring of wastewater and seawater samples
A. Margareto Mato, CSIC-IDAEA / Environmental Chemistry; S. Diaz-Cruz, IDAEA-CSIC / Environmental Chemistry; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research

Keywords: multi-residue, environmental, wastewater, seawater. Antibiotics are one of the pharmaceutical classes with higher usage worldwide. They are both used in human and veterinary medicine, mainly for treating or preventing bacterial infections. After administration most pharmaceuticals are not completely metabolized and thus they are excreted from the body via urine and faeces reaching the wastewater treatment plants via wastewater. Several reports have shown that many antibiotics and their residues are not degraded during wastewater treatment, therefore, discharged to environmental waters. One of the most notorious and significant negative effects attributed to the occurrence of antibiotics in the environment is the development of bacterial antibiotic resistance. While antibiotic-resistant bacteria are found in the natural environment, significantly higher number of these bacteria are present in wastewater or even in treated wastewater [1]. But not only continental water is affected, the use of antibiotics in aquaculture, thus their presence in seawater, has adverse effects on the marine environment as well. This work describes the development, validation and application of a fully automated analytical method for the determination of antibiotics from different therapeutic groups i.e. penicillins, tetracyclines, aminoglycosides, nitrofurans, cephalosporins, macrolides, quinolones, fluoroquinolones, sulphonamides and four of their acetylated metabolites, in wastewater and seawater. The method was based on automated on-line solid-phase extraction (SPE) followed by high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry (HPLC-QqLIT-MS/MS).

Quantification of target antibiotics was performed by the internal standard method and matrix-matched calibration compounds for the different antibiotic groups. The performance of the method in in terms of sensitivity, linear range and precision will be presented. To assess the applicability of the developed method, wastewater and seawater samples were analyzed. The results obtained will be discussed during the presentation. Acknowledgements This work was funded by the EU through the Project Sea on a Chip (Contract 614168) and the Spanish Government (CONVOCATORIA CONSOLIDADO RESEARCH GROUP “2014 SCIR 418 - Water and Soil Quality Unit” References [1] S. Kim, D.S. Aga, J. Toxicol. Environ. Health B: Crit. Rev. 10 (2007) 559

MO248
Fine particulate matters - PM10 in the Novi Sad’s ambient air
D. Ljubicic, Institute of Public Health of Vojvodina; M. Ivancovic, S. Bobic, L. Torovic, Institute of Public Health of Vojvodina / Department of Hygiene and Ecology; N. Dragic, University of Novi Sad Faculty of Medicine; S. Bujelic, University of Novi Sad Faculty of Medicine / Department of Hygiene and Human Ecology

Results from the recent EEA Report from 5/2014 shows that Europe’s air pollution problem is far from solved. One of the Europe's most problematic air pollutants, that one sample (1.30%) exceeded 24-hour PM<sub>10</sub> limit value (50 µg/m<sup>3</sup>), and weig...
PBDEs. Alkylphenols were surveyed in lagoon waters and in dewatering plants. In this case, 4-nonylphenol in lagoon water showed some peaks very close to MAC-EQS. Again, both 4-nonylphenol and octylphenol showed higher concentrations in “mainly urban” dewatering plants than “mainly rural” dewatering plants with some exceptions especially for octylphenol. Integrated analysis of different types of water samples helps understanding sources and fate of contaminants of emerging concern, especially those which are very highly mobile in water and soil. A distinct sharp and peaks which are characterized by manifold antraphoric uses, leading in some occasion to exceed EQS and impair chemical status of water bodies. Both PBDEs and alkylphenols seem characterized by urban origin and maybe found more frequently in stormwater runoff discharges than in industrial and WWTP effluents.

MO251 Loads of parabens, triclosan and triclocarban in greywater: are PCPs the main source of pollution? G. Cekstere, G. Préfecture de Police; E. Caupos, Université ParisEst LEESU UMRMA UPEC; P. Vollenweider, WSL / Forest Dynamics; M. Vighi, University of Milano / Earth and Environment a)

PCPs are not the only source of parabens and triclosan i

MO253 Biomonitoring of Dioxins/dl-PCBs in the north of the Netherlands: eggs of backyard chickens, cow and goat milk and soil as indicators of pollution A. Arkenbout, Toxicowatch Foundation

In the region of Harlingen in the north of the Netherlands people have recently been very concerned about adver health effects possibly caused by dioxins emissions of dioxins and other POPs by a recently installed waste incinerator. To evaluate these effects the local authorities make use of a very limited monitoring program. Using eggs of backyard chickens, cow and goat milk and soil as indicators we however set up a more adequate monitoring program. Because the Dutch authorities do not routinely check the eggs from small farms (or households) – with less than 200 chickens per farm – the risk of pollution with dioxins and dl-PCBs is underestimated. After finding several egg samples that did not comply with the Dutch regulation IKB for the commercial use of eggs (in case the level exceeds 1.75 pg BEQ / g fat one is compelled to take further action) we started a more extended monitoring program. This study shows that eggs of backyard chickens are sensitive biomonitoring parameters for dioxin/PCB contamination in the vicinity of potential sources (harbour activity, waste incinerator, landfill). By using cost-efficient screening analysis tools (such as DR CALUX®) that are also affordable by private households, areas of public concern can be monitored in a rapid and efficient way. The correlation between DR CALUX® and GC/HRMS proved to be very satisfactory; there were no false positive or false negative results. The results of this study point to at least two types of source of dioxin emission near Harlingen. The fact that the levels of dioxin contamination of backyard chickens are increasing with decreasing distance to the harbour urgently calls for closer investigation in order to find the sources of the dioxin/PCB pollution, and to prevent adverse health impacts for domestic animals, wildlife and human beings.

MO254 POPs and Pseudo-POPs: Environmental occurrence and exposure (P)

Scientific rationale for the evaluation of PBT and POP criteria and cut-off values in international regulatory tools M. Matthaei, University of Guelph / School of Environmental Sciences; K. Solomon, University of Guelph / School of Environmental Sciences; M. Vighi, University of Milano / Earth and Environmental Sciences; A. Gyilman, Sustainable Solutions International; J. Tarazona, EFSA

The concepts of PBT and POP assessment are extremely important for regulatory purposes because they allow identifying and classifying substances that require particular attention for the control of chemicals on the market (e.g. PBTs or POPs in REACH) or that need to be banned or strictly controlled at global level due to their potential for long range transport (L) and to become global pollutants (e.g. POPs in the Stockholm Convention). In spite of this internationally recognised relevance for regulators and policy makers, these concepts are not fully consistent in international regulatory tools and their meaning is changing. In particular, the origin of the criteria and the evolution of their cut-off values and the specific testing requirements often do not have a clear and identifiable scientific rationale. An overview is given on the different definitions used to identify these kinds of chemicals. Most national regulations and international agreements derived cut-off values from substances with known PBT/POP or POP properties (“dirty dozen”) by using them as reference chemicals. In particular, differences in the cut-off values for P, B, T and L concepts are highlighted and the consequences of these differences (e.g. the different classification and the different number of chemicals requiring strict control measures) are discussed. The need for using sound science-based criteria or precautionary-based values required for management-policy reasons is also accounted for. The possibility of rationalization of approaches is discussed in connection with the different protection aims of the various regulatory frameworks.

MO255 A pilot study on the exposure of individuals to chemical pollutants, via ritualistic consumption of Ganges River Water, during the Maha Kumbh Mela (2013). B. C. Lemanski, Department of Biology; S. Gullapalli, Colgate University / Department of Economics; S. P. Connor, Wadsworth Center New York State Department of Health / Department of Analytical Chemistry

The Maha Kumbh Mela is one of the most important religious pilgrimages in Hinduis

MO256 Impact of road de-icing salt on foliage of ornamental lime trees (Tilia x vulgaris H.) in the city of Riga (Latvia) G. Cekstere, G. Préfecture de Police; E. Caupos, Université ParisEst LEESU UMRMA UPEC; P. Vollenweider, WSL / Forest Dynamics; M. Vighi, University of Milano / Earth and Environment a)

Dioxins and dioxin-like PCBs are still poorly understood. Objectives were to compare levels of de-icing salt (NaCl) contamination in street tree leaves with the concentrations of nutrients and micronutrients in stormwater that appeared as concentrated and contaminated greywater were sampled: manual dishwashing, washing machine, showers and washbasin. Eight compounds were analyzed: methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), benzylparaben (BzP), butylparaben (BuP), isobutylparaben (iBuP), TCS and TCC by liquid chromatography coupled with tandem mass spectrometry. Concentrations and loads in µg/inhabitants/day were assessed. For MeP, the most concentrated compounds, concentrations vary from 40 ng L⁻¹ to 2.1 mg L⁻¹ leading to a median flux of 500 µg/inhabitant/day that represents around 16 % of the loads carried by wastewater. The key lessons highlighted by this study are that greywater strongly contributes to the contamination of wastewater. However PCPs are not the only source of parabens and triclosan in wastewater: dishwashing and manual dishwashing as shower and washbasin. Moreover measured concentrations were highly variable depending on the consumption practices. This latter finding indicates the need to link environmental sciences with social sciences; this is our outlook for a future research in the frame of the Cosme' eu project. New samplings of wastewater will be carried out in 2015 to evaluate the evolution of concentrations in wastewater in relation to potential conservative and bioaccumulative factors for parabens (e.g. use per person/week, body weight). Keywords: Greywater, loads, personal care products, paraben, triclosan. ʌn

Impact of road de-icing salt on foliage of ornamental lime trees (Tilia x vulgaris H.) in the city of Riga (Latvia) G. Cekstere, G. Préfecture de Police; E. Caupos, Université ParisEst LEESU UMRMA UPEC; P. Vollenweider, WSL / Forest Dynamics

On the other hand they may indicate simultaneous uptake of salt and other environmental contaminants. Negative correlation between salt and Ca, B and Mg concentration may relate to injury in mesophyll cells caused by NaCl accumulation. Given the sensitivity of lime trees to road de-icing salt, accumulation of NaCl at sensitive - e.g. mesophyll – sites, where they may severely affect cell physiology and cause structural injury, is expected.

MO254 POPs and Pseudo-POPs: Environmental occurrence and exposure (P)

Scientific rationale for the evaluation of PBT and POP criteria and cut-off values in international regulatory tools M. Matthaei, University of Guelph / School of Environmental Sciences; K. Solomon, University of Guelph / School of Environmental Sciences; M. Vighi, University of Milano / Earth and Environmental Sciences; A. Gyilman, Sustainable Solutions International; J. Tarazona, EFSA

The concepts of PBT and POP assessment are extremely important for regulatory purposes because they allow identifying and classifying substances that require particular attention for the control of chemicals on the market (e.g. PBTs or POPs in REACH) or that need to be banned or strictly controlled at global level due to their potential for long range transport (L) and to become global pollutants (e.g. POPs in the Stockholm Convention). In spite of this internationally recognised relevance for regulators and policy makers, these concepts are not fully consistent in international regulatory tools and their meaning is changing. In particular, the origin of the criteria and the evolution of their cut-off values and the specific testing requirements often do not have a clear and identifiable scientific rationale. An overview is given on the different definitions used to identify these kinds of chemicals. Most national regulations and international agreements derived cut-off values from substances with known PBT/POP or POP properties (“dirty dozen”) by using them as reference chemicals. In particular, differences in the cut-off values for P, B, T and L concepts are highlighted and the consequences of these differences (e.g. the different classification and the different number of chemicals requiring strict control measures) are discussed. The need for using sound science-based criteria or precautionary-based values required for management-policy reasons is also accounted for. The possibility of rationalization of approaches is discussed in connection with the different protection aims of the various regulatory frameworks.

MO255 A pilot study on the exposure of individuals to chemical pollutants, via ritualistic consumption of Ganges River Water, during the Maha Kumbh Mela (2013). B. C. Lemanski, Department of Biology; S. Gullapalli, Colgate University / Department of Economics; S. P. Connor, Wadsworth Center New York State Department of Health / Department of Analytical Chemistry

The Maha Kumbh Mela is one of the most important religious pilgrimages in Hinduis

MO256 Impact of road de-icing salt on foliage of ornamental lime trees (Tilia x vulgaris H.) in the city of Riga (Latvia) G. Cekstere, G. Préfecture de Police; E. Caupos, Université ParisEst LEESU UMRMA UPEC; P. Vollenweider, WSL / Forest Dynamics

On the other hand they may indicate simultaneous uptake of salt and other environmental contaminants. Negative correlation between salt and Ca, B and Mg concentration may relate to injury in mesophyll cells caused by NaCl accumulation. Given the sensitivity of lime trees to road de-icing salt, accumulation of NaCl at sensitive - e.g. mesophyll – sites, where they may severely affect cell physiology and cause structural injury, is expected.
collected whole water samples (water and suspended sediments) at six sites of religious significance spanning the length of the river - Devprayag, Rishikesh, Haridwar, Allahabad, Varanasi, and Gangasagar - from December 2012 to February 2013. These samples were subsequently analyzed for select polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs), and polycyclic aromatic hydrocarbons (PAHs), through micro-electrode capture or mass spectrometric methods as were applied. Results suggest that while previous studies may have found high levels of pollutants, the levels of surveyed chemical pollutants were relatively low at all sites surveyed.

MO258 Polychlorinated Biphenyls Monitoring in Surface Water

A. Aleksandryan, Hazardous Substances & Waste Policy Division / Head of Division; A. Khachatryan, Waste Research Center; B. Gabrielyan, Waste Research Center; Y. Bunyatyan, Waste Research Center

Keywords: PCBs, Surface Water, Production, Distribution

Among major sources of environmental pollution are power transformers, turbines and other type of equipment filled with polychlorinated biphenyls (PCBs)-containing liquids, as the mentioned equipment is functioning in the industry, power engineering and other branches of National Economy in a considerable quantity. The daily risk of PCB-related affections in big groups of population in the course of professional activity of humans is extremely great. The greatest danger to environment and people originates from incompetent handling of these compounds, ignorance among owners of PCB and PCB-filled equipment about the hazards of the given substances, as well as contact to soils, foodstuff and other objects polluted by PCBs. Monitoring of PCBs was performed on samples of water from SevernLakand rivers of its basin, biomedia of the lake and rivers of its basin, bottom sediments, soil near the former pesticides shops and storages, as well as a black soil in the vicinity of these shops and storages, area formerly known by pesticides intense application In 2002-2003, relatively high amounts of PCBs were revealed in samples from rivers flowing near major settlements: inGavagarRiver– 0.751 mcg/L, inMartuniRiver– 1.577 mcg/L (Table 3.1.4). In these rivers besides the impact of agricultural runoffs, residential, household-related pollution is added. In 2012 Samples of surface water from SevernLakand rivers of its basin, benthos, biotopes and other type of soil from Severn and rivers of its basin, as well as soil samples from different regions were taken. The average water samples were studied using Gas-Chromatography. Totally 46 samples were analyzed for PCB and 596 findings were obtained. Average PCBs content in water fromSevernLakamede 0.727 mcg/L, while the maximum level was 6.831 mcg/L. In biomedia the average level of PCBs was 0.530 mcg/L and the maximum content was 0.743 mcg/L. Upon investigation of the mentioned environmental media a typical picture of technogenic pollution by PCBs due to application of transformer oils of “Arochlor” type was observed. To this latter signifies the content of PCB revealed congeners. Furthermore, attention should be paid to the presence of great amounts of congeners No.87 and No.95 among all the revealed PCB congeners; this also confirms the fact that transformer oils are the source of pollution. The research outcomes demonstrated that, generally, PCBs content did not exceed the hygienic standard.

MO259 Input of neonicotinoids into the aquatic environment after application as seed treatment

E.K. Nam, Institute for Sustainability Sciences ISS; M.F. Garcia Delgado, Agroscope / Institute for Sustainability Sciences ISS; I. Hanke, Plant Protection Chemistry; S. Hutsona, Agroscope / Plant Protection Chemistry; R. Kasteel, T. Poiger, Agroscope; T.D. Bucheli, Agroscope ART / Analytical Chemistry Natural Resources Environmental Protection in Agriculture

Today, modern agriculture is not feasible without the use of plant protection products. Since 1997, the neonicotinoids (Imidaclopid, Thiamethoxam) as well as the other type of soil from Severn and rivers of its basin, as well as soil samples from different regions were taken. The average water samples were studied using Gas-Chromatography. Totally 46 samples were analyzed for PCB and 596 findings were obtained. Average PCBs content in water from SevernLakamede 0.727 mcg/L, while the maximum level was 6.831 mcg/L. In biomedia the average level of PCBs was 0.530 mcg/L and the maximum content was 0.743 mcg/L. Upon investigation of the mentioned environmental media a typical picture of technogenic pollution by PCBs due to application of transformer oils of “Arochlor” type was observed. To this latter signifies the content of PCB revealed congeners. Furthermore, attention should be paid to the presence of great amounts of congeners No.87 and No.95 among all the revealed PCB congeners; this also confirms the fact that transformer oils are the source of pollution. The research outcomes demonstrated that, generally, PCBs content did not exceed the hygienic standard.

MO260 Multi-stressor modeling - assessment of D5 (decamethylocyclopentasiloxane)

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behavior in the Baltic Sea using the next generation multimedia fate and transport model BALTSEM-POP

E. Undeman, Stockholm University / Dept of Applied Environmental Sc; B. Gustafsson, Stockholm University / Baltic Nest Institute; Baltic Sea Centre; C. Humborg, Stockholm University / Department of Applied Environmental Science

ITM Baltic Sea Centre; M.S. McLachlan, Stockholm University / Department of Environmental Science and Analytical Chemistry; CES

Organic contaminants constitute one of many stressors that deteriorate the ecological status of the Baltic Sea. When managing environmental problems in this marine environment it may be necessary to consider the interactions between various stressors to ensure that averting one problem does not exacerbate another. A novel modeling tool, BALTSEM-POP, is presented here that simulates interactions between organic contaminants, biogeochemical cycling, deposition, and transport in the Baltic Sea. We discuss opportunities to use the model to support different aspects of chemicals management. We exemplify these opportunities with a case study where two emission reduction strategies for a chemical used in personal care products (decamethylcyclopentasiloxane) are evaluated, and where the confounding influence of future climate change and eutrophication on the impact of the emission reduction strategies are assessed.

MO261 Spatio-temporal pattern of pesticide residues in the Turia and Júcar Rivers (Eastern Mediterranean Area of Spain)

A. Granuel, C.D.E. Valencian CSIC; P. Cid, CsIC UV; G. Andreu, U.V. University / Medicine Preventive; a.m. reyes; V. Andreu, CIDE CSIC UV GV; Y. Pico, University of Valencia / Medicine Preventive

The Júcar and Turia rivers, with a length of 512 and 280 km respectively, are the most important of the Júcar hydrographic demarcation (East of Spain). The main fluvial network has a markedly Mediterranean water contribution regime, characterized by dry periods in the summer and by growth of circulating flow in the fall. The average annual rainfall is 500 mm; however, there is large spatial variability with values of 300 mm in the southern regions, whereas in other areas reached values are greater than 750 mm. The Júcar hydrographic demarcation contains large crop areas in which pesticides are applied widely to protect plants from disease, weeds and insects. This work presents the results of analyzing 50 commonly-used pesticides by liquid chromatography of 81 water and sediment samples taken from both rivers and in consecutive periods of time as follows: 30 samples from the Júcar river in the period 2010-2011, and 51 samples from the Turia river in the period 2012-2012. The study also presents the results of correlating pesticide concentration and physical-chemical characteristics of the water and sediment matrices. In the two rivers basins, pesticides were detected primarily in water (Júcar 58% and Turia 92%), whereas their presence in sediments was more intermittent. Those at high concentrations in water were the azole (imazalil up to 6750 ng L⁻¹), the organophosphorus (chlorpyriphos in 100% of the samples), and the Benzimidazole (carbazendin up to 382 ng L⁻¹). However, this pattern differed in sediments, which were contaminated primarily with organic contaminants higher than chlorpyriphos 100% of sediments in 2010-2011 and 64% in 2012-2013 up to 141 ng g⁻¹. According to the results of this study, pesticide residues in the Júcar and Turia basins do not seem to represent a high risk to sediments. Nevertheless, the monitoring program can be very useful to control the contamination of the river basin. Acknowledgement – The authors thank the Spanish Ministry of Economy and Competitiveness for the financial support through the projects Consolider-Ingenio 2010 CSD2009, and CGL2011-29703-C02-02.

MO262 Spatio-temporal patterns of selected organic pollutants in sediments from western Svalbard fjords

K. Pawling, A. Pouch, Institute of Oceanology Polish Academy of Sciences-Centre for Polar Studies; A. Zaborska, Institute of Oceanology Polish Academy of Sciences; g. siedlewicz, IO PAN / Marine Chemistry and Biochemistry Department

The knowledge of biogeochemical cycling of organic contaminants in polar marine ecosystems is still incomplete. Meanwhile, knowledge of migration routes of these compounds and factors determining their distributions in a given ecosystem is essential to the reliable determination of ecological and ecotoxicological risks arising from the presence of these compounds in the ecosystem. Moreover, polar ecosystems are considered to be particularly sensitive to changes in environmental factors related to climate change. Global warming may cause changes in pollution load to the Arctic seas as well as affect the transfer of contaminants between biotope and surrounding ocean and atmosphere. The aim of the study was to examine the final sink for important portion of organic pollutants entering the Arctic, thus can be used as a useful tool in reconstruction of pollution history. In the present study we examine the contaminant record in two Svalbard fjords – Hornsund and Kongsfjorden using sediment cores dated by 137Cs geochronology. Sediments cores were collected during in r/v “Oceania” Arctic campaigns. Composition and levels of persistent organic contaminants (2,3,7,8-TCDD, 2,3,7,8-PCDD/Fs, and 2,3,7,8-PCB) in these layers were investigated. Selected samples are analysed for the presence of pharmaceutical residues. Potential POPs sources were identified based on interpretation of the congener proportions and overall sediment concentrations of the studied compounds. The study also provides an opportunity to discuss the influence of burial and post-depositional sediment reworking processes on the interpretation of persistent organic contaminants detected in marine sediments. Finally the obtained results provide good basis to predict future changes in organic pollutants cycling in changing Arctic environment. The presented study was partly realised in the frame of Centre for Polar Studies (Leading National Research Centre)

MO263 Depositional history of polychlorinated biphenyls (PCBs) in two South Atlantic subtropical estuarine systems

T. Combi, University of Bologna / Environmental Sciences; S. Taniguchi, University of Sao Paulo / Physical Oceanography department; C.D. Martins, Universidade Federal do Rio Grande do Sul / Centro de Estudos do Mar

The environmental patterns and fate of persistent organic pollutants (POPs) are of global concern because of their high persistence in the environment, liposolubility, capacity of biomagnification and endocrine disrupter and carcinogenic characteristics. Considering several factors, such as chemical stability and extensive global usage, polychlorinated biphenyls (PCBs) are often used to assess the environmental behavior of POPs. To assess the spatial and temporal distribution of PCBs, sediment cores and surface sediments from two South Atlantic subtropical estuarine systems (Guararuba and Paranaguá Bays) were analysed. The study area is located in the Southeastern Brazilian coast and is surrounded by one of the last preserved Atlantic Rainforests of South America. While Guararuba Bay represents a well-preserved environment and has been selected by the Nature Conservancy’s Coasts and Marine Conservation Program as a priority site for biodiversity conservation in Brazil, Paranaguá Bay is surrounded by urban and industrial areas and shelters the largest grain exporter port in Latin America. The historical input of PCBs in the area was elucidated through the integrated analysis of the vertical profile and sedimentation rates. Since PCBs were not produced in Brazil, historical trends show a late usage in comparison with industrialized areas from Northern Hemisphere; PCBs were not detected before the decade of 1960 and the first peak of relative high concentration occurred around 1965. The next decades were characterized by increasing concentrations of PCBs, reaching the highest values between the late 1980’s and beginning of 1990’s, which corresponds to the period of increased human occupation around the region. Concentrations decreased in recent years, reflecting the prohibition of the use, production and importation of PCBs since 1981. Distribution of PCBs in Paranaguá Bay showed the anthropogenic activities (port, industries and urbanization) on the margins of the bay are the major responsible for the contamination in the area. In contrast, the main source of PCBs in Guararuba Bay was located outside the estuary, with PCBs coming through the rivers that flow into the bay. Notwithstanding the early ban on PCBs production, urban and industrial emissions are predicted to continue in the next decades, highlighting the importance of understanding the sources and distribution of these compounds worldwide.

MO264 Historical sediment record and distribution of polychlorinated biphenyls (PCBs) in sediments from the Adriatic Sea (Italy)

T. Combi, University of Bologna / Environmental Sciences; S. Misericocchi, L. Langone, CINR / Institute of Marine Science ISMAR; R. Guerra, University of Bologna / Department of Physics

PERSEUS EU FP7 Project (Policy-oriented marine Environmental Research in the Southern European Seas) aims to identify the interacting patterns of natural and human-processed pressure to the Mediterranean Sea and their impact on marine ecosystems in the framework of the Marine Strategy Framework Directive (MSFD). In the context of the ‘ADREX: Adriatic and Ionian Seas Experiment’ within the PERSEUS project, a monitoring survey has been conducted in order to investigate spatial and temporal patterns of polychlorinated biphenyls (PCBs) in sediments from the Adriatic Sea (Italy). To this end, surface sediment along transversal-to-the-coast transects and undisturbed sediment cores were collected at four coastal areas: the prodelta Po River, Ancona, Gargano Promontory and Bari. The spatial distribution of total PCB concentration in surface sediments varied between 4.1 kg kg⁻¹ and 0.84 kg kg⁻¹, and revealed a decreasing pattern from the northern to the southern Adriatic Sea. The maximum concentration of total PCBs was detected in the Po River prodelta area, and the lowest concentrations were found in the Bari coastal area. The historical trends of PCBs showed a common pattern, with increasing concentrations from the lower horizons to the middle sections of sediment cores, followed by a decreasing trend upwards in the surface layers deposited in recent years. Historical data on PCBs show that production and emissions increased sharply from the mid 1960’s to 1980’s in Italy, to decrease in the following decades. Concentrations found in middle sections of sediment cores probably recorded periods of higher production and emission of PCBs, while the lower concentrations found in the upper layers most likely reflected ban/restriction on PCB production and use in Italy due to incoming European regulations. Despite the low levels of total PCBs found in surface and middle sections of sediments when compared to highly urbanized coastal areas in the World, concentrations have declined with distance from the Po River prodelta southward, suggesting the Po River outflow to be a major contributor of PCBs to sediments in the Adriatic Sea. In addition, the presence of PCBs in areas far from the direct source of input and the congeners pattern suggest that PCBs in the sediments from the Adriatic Sea may also come
from discharges of local sources, as well as atmospheric deposition.

**MO265**

**Sources and of Polychlorinated Biphenyls (PCBs) in Admiralty Bay, King George Island, Antarctica**

T. Combi, University of Bologna / Environmental Sciences; C.d. Martins, Universidade Federal do Parãu / Centro de Estudos do Mar; S. Taniguchi, Universidade de Sao Paulo / Physical Oceanography department; J. Leoni, Universidade Federal do Rio Grande / Instituto Oceanográfiico; R.A. Lourenêo, PUCRío; R.C. Montone, Instituto Oceanográfiico - Universidade de Sao Paulo / Departamento e Oceanografia Física Química e Geológica

Long-range transport of persistent organic pollutants (POPs) to polar regions is one of the most important actual environmental problems. Even though the Antarctic continent is the most remote and protected area on the planet, recent studies suggest that it is no longer a pristine environment, being subject to both direct impacts due to increasing tourism and research activities and indirect impacts through atmospheric transport. Sediment cores were analysed for temporal patterns and sources of polychlorinated biphenyls (PCBs) in Admiralty Bay, King George Island. The samples were taken at nine sites: Ferraz Station; Ullman Point; Botany Point; Stenhousen Point; Refuge II; Crepin Point; Arctowski Station; Barrel Point; Thomas Point. Total PCB concentrations were slightly higher in comparison with other remote and well-protected areas, ranging from 1-Idry weight. In a general way, the historical distribution of PCBs presented relatively high values around the middle of the decade of 1980, coinciding with the installation of research stations and increased tourism activities in the study area. However, this trend was not verified for all studied locations and some of the sediment cores registered non-negligible PCB concentrations in surface sediments, corresponding to the period after the year 2000. The detectable levels in recent sediments and the lack of a clear decreasing trend on PCB concentrations in the majority of the sediment cores can be a result of several factors, such as emissions from current sources and local land use changes in different countries. In addition, previous studies have shown that the dynamics of changes of the glacier barriers can affect the concentrations and represent a source of organochlorine contaminants to polar marine ecosystems. The similar congener composition and the prevalence of low-chlorinated PCBs indicate same source of PCBs and confirms long-range transport is the most likely source of PCBs to the Antarctic environment. In addition, the presence of some high-chlorinated PCB points out to a minor influence of local input sources, especially in the sediment core collected close to the Brazilian Station Comandante Ferraz. This study provides valuable information concerning the contamination status of the Antarctic continent, confirming the increasing impact of anthropic pressures and long-range transport of pollutants in remote environments.

**MO266**

**Influence of soil organic matter on the PAHs accumulation in the top layer of agricultural land of different anthropopressure**


Incorporation of organic matter into the soil is a key factor in determining the pattern of chemical behavior and the persistence of some contaminants (e.g. polycyclic aromatic hydrocarbons (PAHs)) in soils. The organic matter content of the soil is key factors to determinate the degradation rate of some organic contaminants and the way in which they are released into the environment. Nowadays, enhanced transformation of contaminants into “bound” residues (polychlorinated biphenyls) by microorganisms, heterotrophic activity; however, this activity could be modified through the variation of physical, chemical or biological factors. Many studies have shown that parameters like the temperature, the organic matter content and the acidity of the soil are key factors to determinate the degradation rate of some organic contaminants and the way in which they are released into the environment. Therefore, the role of the soil organic matter in the fate of some contaminants is one of the key points in the understanding of the behavior of these contaminants in the environment. In this study, the possible effects of the soil organic matter on the fate of PAHs in the top layer of agricultural land of different anthropopressure were studied. The study was performed in the region of Lombardy, Italy, where the activities associated with the production of energy based on fossil fuels have caused an accumulation of PAHs in the soil. The aim of the study was to evaluate the influence of the soil organic matter on the fate of PAHs in the top layer of agricultural land of different anthropopressure. The results showed that the soil organic matter content is a key factor in determining the degradation rate of some organic contaminants and the way in which they are released into the environment.

**MO267**

**Biodegradation of pesticides in soil under different environmental conditions**

A. Muskus, UFZ Center for Environmental Research / Department Umweltbiotechnologie; K.M. Nowak, RWTH Aachen University / Institute for Environmental Research Biology; V. Hanner, WWU - University of Muenster / Institute of Landscape Ecology; M. Kerkow, Helmholtz centre for environmental research - UFZ / Department of Environmental Biotechnology.

Anthropogenic organic chemicals like pesticides (2,4-Dichlorophenoxyacetic acid and Glyphosate) are deliberately released in major amounts to nearly all compartments of the environment. Soil as a complex matrix provide a wide variety of binding sites and are the major sinks for these compounds. Xenobiotics entering the environment may undergo various processes like transformation, degradation, volatilization, sorption and leaching which determine their fate and potential environmental impact. Therefore, the understanding of the biodegradation processes and the factors influencing them is crucial to assess the persistence of these chemicals in the environment. This study aimed to investigate the biodegradation of pesticides in soil under different environmental conditions. The results showed that the biodegradation of pesticides is significantly influenced by environmental factors such as temperature, moisture, pH, and oxygen availability. Consequently, the biodegradation processes may vary depending on the environmental conditions, and these changes should be considered in the risk assessment and management of pesticides in the environment.

**MO268**

**Comparing spatial variability of PCBs, PCDD/Fs and metals in heavily contaminated farm soils in Brescia (Italy)**

A. Di Guardo, University of Insubria / Department of Science and High Technology; G. Raspà, La Sapienza University / Department of Chemical Engineering Materials and Environment; S. Borin, University of Milan / DeFENS; E. Zanardini, C. Morosini, University of Insubria / DSAT; S. Armiraglio, Municipality of Brescia / Museum of Natural Sciences; V. Sale, S. Anelli, P. Nastasio, ERSAF

The Brescia Calfaro is a national priority polluted site located in Brescia, a city of about 200,000 inhabitants in northern Italy. The site originated from the activities of the former Calfaro s.p.a. chemical factory, the largest former PCB producer in Europe, which produced such chemicals for more than 50 years up to mid 80’s. About 100 Ha of agricultural soils in the SIN Brescia Calfaro present a mixed contamination of halogenated Persistent Organic Pollutants (POPs, mostly - polychlorinated biphenyls) and heavy metals (Hg, As) in variable concentrations, often exceeding the safety values. Contamination by Polychlorinated biphenyls (PCBs) is of particular relevance, since they were classified as carcinogenic, are extremely persistent in the environment and bioaccumulate and biomagnify in the food web. The clean-up of this site is therefore a priority for local (Lombardy Region, Brescia municipality) and national authorities. Among the actions planned, a specific research program started in 2014, to test pilot bioremediation processes to be later applied to the entire agricultural area. Given the extension of the contaminated site the application of biologically-mediated techniques is among the sustainable choices available. The use of plants to extract and modify the pollutants (phytoremediation) together with root associated microbes to i) degrade organic pollutants or modify the heavy metals (rhizoremediation), or ii) stimulate plant growth (plant growth promotion, PGP) is a promising activity for the SIN Brescia Calfaro bioremediation. However, the field-scale application for POP bioremediation has nevertheless few preceding studies and in particular needs a multidisciplinary approach for the characterisation of remediation potential, and monitoring and modelling of the process. In this part of the research, the activities focused on the field-scale application of the biological degradation by atmospheric deposition as well as surface water transport and subsequent redistribution in space are reported with the aim to guide following bioremediation. A three phase analytical campaign was started to identify the contaminants, basing on the historical factory production inventory in order to evaluate the spatial distribution (both horizontal and vertical) in three different areas and plan future actions. Acknowledgements: The project is funded by Lombardy Region, within the remediation activities of the SIN Brescia Calfaro.
2; orphans: 2; In the long term soils generally act as sinks for airborne persistent organic pollutants (POPs), which were released into the environment by natural sources and anthropogenic activities. Diurnal temperature changes, however, may vary the source-sink function of soils for atmospheric pollutants. As a result, POPs cycle between soils and the atmosphere. In this study, we applied an extended version of the multicomponent reactive transport code MIN3P to model the short-term concentration evolution of gas-phase pollutants in the atmospheric boundary layer over a time period of three days. Phenanthrene was chosen as representative chemical for many semi-volatile POPs. We assumed two systems: a closed system that neglects mass exchange of gaseous phenanthrene with the upper atmosphere, and an open system that allows mass exchange with the upper atmosphere. For each system, we modelled the concentration evolution of gaseous phenanthrene with deposition on particulate matter (PM, soil depth) and with respect to different heights of the atmospheric boundary layer. Phenanthrene was affected by photo-degradation in the atmosphere during daytime, and by biodegradation in the soil compartment at all times. We found that diurnal temperature changes in the topsoil cause concentration fluctuations of POPs in the atmosphere. Due to strong retardation of POPs in the soil compartment, the magnitude in concentration evolution in the soil depends on temperature changes, regardless of the prevailing atmospheric conditions and the height of the atmospheric boundary layer. In contrast, in the atmosphere, the magnitude in concentration evolution is determined by the atmospheric conditions and the height of the atmospheric boundary layer. Moreover, a phase shift in peak concentration in the atmosphere with respect to the atmospheric temperature evolution was observed. However, the distance between the plant and the source is a crucial element in the partitioning of POPs between soil and the atmosphere. The concentration gradient forming in the atmosphere can be taken as an index to identify the source-sink function of soils for airborne pollutants. Our model results can be applied to interpret the diurnal pattern for the concentration evolution of atmospheric pollutants. Also, the long-term environmental fate of POPs between soil and the atmosphere can be evaluated.

MO270 Past vs. recent sources of PCBs in Italy: an analysis of soil and air concentrations in four transects from a large remediation site E. Terrazghi, University of Insubria (Como) / Department of Science and High Technology; T. Mercurio, University of Insubria Como / Department of Science and High Technology; G. Raspa, La Sapienza University / Department of Chemical Engineering Materials and Environment; A. Di Guardo, University of Insubria / Department of Science and High Technology

PCBs are a family of 209 compounds (congeners) widely produced and used in many countries for many decades until their production was banned. PCBs, being high potential contaminants, tend to accumulate in the food chain, posing adverse effects to the human health and the environment. Even if measures were taken to reduce their release to the environment and their environmental levels experienced a substantial decrease, these compounds are still present in the environment and can be released from reservoir compartment or secondary sources, such as contaminated sites. The area of Brescia, a highly industrialized city in the Lombardy Region (Northern Italy) is characterized by the presence of a Site of National Interest (SIN) for remediation (SIN Brescia-Caffaro). Here, an industrial plant (Caffaro S.p.a.) produced PCBs for about 50 years (1930–1984) and its surrounding areas were found to be heavily contaminated with high concentrations in air, irrigation water and soils at mg/kg levels. The aim of the present study was to investigate the potential of the contaminated area in driving the PCB contamination in the surrounding areas and the current effects on air concentrations. Different sampling campaigns were organized to collect samples of soil and leaves along four ∼100 km transects that ran in NW, NE, SW and SE directions considering the production plant as starting point. 16 samples were collected for each transect at about 7 km distance. In each sample the following PCB congeners were determined employing GC-ECID: tri-PCB 28; tetra-PCB 52; penta-PCB 101, hexa-PCB 153, 158; hepta-PCB 180; Deca-PCB 209. The results of each transect were compared in order to evaluate the presence of a contamination gradient, that decrease with increasing distance from the point source. Moreover the fingerprints of the soil and leaf samples were compared to profiles of soils from the contaminated site in order to understand if the concentrations found in the transect samples could be attributed to a significant contaminant in the food chain. Results revealed that the data produced in order to evaluate the gradients, also considering weathering phenomena which could have occurred in time altering the original PCB congener profiles. A number of simulations were also performed using a dynamic air-vegetation-soil model (SoilPlusVeg) in order to interpret the actual soil and leaf concentrations and predict the order of magnitude of fluxes.

MO271 Evaluation of models for gas-particle partitioning of polycyclic hydrocarbons in background air in central Europe P. Shalpour, Max Planck Institute for Chemistry / Multiphase Chemistry; G. Lammel, Masaryk University, A. Holubová Smekalová, Czech Hydrometeorological Institute; J. Klanova, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; L. Landlova, P. Pribylova, Masaryk University; M. Váha, Czech Hydrometeorological Institute

Gas-particle partitioning is an important mechanism, which affects the transport and fate of semi-volatile organic compounds (SOCs). The preferential partitioning of SOCs in the atmosphere depends on parameters such as the compound’s molecular structure as well as particulate matter (PM) physical and chemical properties, and can be explained by various empirical and theoretical models. These include, but not limited to, Junge-Pankow (JP), Harner-Bidleman (HB), Lohmann-Lammel (LL), and poly-parameter linear free energy relationship (plLFER) models. To explain the SOC partitioning, each model considers one or more of the compound’s physico-chemical properties or PM characteristics. Despite the past efforts in determining the most appropriate model, discrepancies remain, as each model neglects certain intermolecular interactions. The aim of the present research was to determine what model better predicts the gas-particle partitioning behavior of polycyclic aromatic hydrocarbons (PAHs) in non-urban environments. To this end, 162 air samples (gas and particulate phase) were collected from Košice observatory, a background site located in central Europe. Summation of PAH concentrations in gas and particulate phase ranged from 0.6 to 138.6 (mean ± standard deviation: 11.3±15.7 ng m⁻³ and 0.1 to 189.3 (9.3±21.2) ng m⁻³, respectively. Median particulate mass fractions for individual PAHs ranged from 0.01 to 0.99. Significant negative correlation was found between the particulate mass fractions and near-ground temperature at the site, highlighting the effect of temperature on SOC partitioning behavior. Initial results showed that HB and LL models are overall better in predicting PAH gas-particle partitioning; however, plLFER could do a more accurate prediction for some individual congeners.

Keywords: semi-volatile organic compounds, polycyclic aromatic hydrocarbons.

MO272 Musk fragrances, among other organic molecular tracer compounds, in urban in-and outdoor particulate matter. B.L. van Drougoe, J.O. Grimalt, Environmental Chemistry

This work presents the analysis of mussk fragrances, among other organic molecular tracer compounds in atmospheric particulate matter (PM) samples collected at indoor and outdoor site in the urban area of Barcelona, including bars, subway platforms, class rooms, playgrounds, road sites and urban parks. PM samples were collected on quartz microfiber filters by means of a high volume sampler and analysed following a described procedure. Galaxolide, tonalide and methyl-dihydrosjamonate were detected in the samples, among other organic compounds, including toxic compounds, such as PAH, but also source-specific compounds, such as those from traffic, tobacco smoke, wood burning, plastics and dust. Indoor environments exhibit higher concentrations of musk fragrances (in ng/m³ to μg/m³ range), and were often correlated with other ‘urban-life-style’ compounds, such as nicotine, from cigarette smoke, hopanes from traffic, or plasticizers from furniture and building material. Outdoor levels were two orders of magnitude lower in urban background sites, such as urban parks and playgrounds, while similar to indoor levels were detected in outdoor air busy street site in the urban center.

MO273 Presence of persistent organic pollutants in urban air: gas-particle partitioning B. Barbas, CIEMAT; A. de la Torre, Environmental; P. Sanz, I. Navarro, M. Martínez, B. Artiñano, CIEMAT

Persistent Organic Pollutants (POPs), and emerging pollutants as flame retardants, are some of the wide extent of dangerous chemicals which can be found in the ambient air. They are present in the gas phase and also adsorbed on the suspended particulate matter, depending on ambient temperature and the physico-chemical properties of the pollutant. Due to their toxic, bio-accumulative and detrimental characteristics these chemicals are of great concern for human health and natural ecosystems. However there are several data gaps related to their fractionation in ambient air. Therefore improving our knowledge of POPs in this matrix remains a challenge. The aim of this study was to evaluate the presence of several POPs in ambient air samples from the city of Madrid (Spain). Gas phase and airborne particulate matter were studied separately. Levels and fractionation of the following analytes were evaluated: polychlorinated dibenz-p-dioxins and dibenzofurans (PCDD/Fs: the 2,3,7,8-substituted congeners), polychlorinated biphenyls (PCBs: dioxin-like PCBs and indicator PCBs), polychlorinated diphényl ethers (PBDEs: tri to deca-BDE) and Dechloranes (Dec 602, Dec 603, Dec 604, Dec 605). The results revealed p-PCBs and PBDEs together as the most dominant classes. Results revealed p-PCBs as indicators for the most polluted, followed in decreasing order by di-PCBs, PBDEs, DP and finally by PCDD/Fs. PBDEs were mainly related to gas phase, while PCDD/Fs, DP and PBDEs were associated with particulate matter. Mirex and Dec 602 were only occasionally detected, while Dec 603, Dec 604 and CP were not detected in any of the analyzed samples. The data obtained in this study concerning the occurrence and concentration profiles of the various analytes evaluated, represent an important advance in the study of the behaviour of these pollutants, resulting very interesting for research on human exposure to POPs.

MO274 Assessment of external routes for human exposure to phthalate plasticizers in indoor environment. A case study based on a Norwegian cohort of 61 adults in Oslo area. G. Giovantonius, IVL Swedish Environmental Research Institute Ltd / IVL Swedish Environmental Institute; J. Magner, A. Palm Cousins, IVL Swedish Environmental Research Institute Ltd; C.A. de Wit, Stockholm University / Applied Life Science
Environmental Science ITM

The extensive use of phthalate esters (PEs) as plastic additives in a wide range of consumer products has caused these contaminants to be ubiquitous in environments relevant for human exposure, and as a result also in human tissues. The main purpose of this study was to investigate the most relevant exposure pathways for human body burdens to phthalate plasticizers. The targeted substances were measured in indoor air in samples from 61 homes in the Oslo area. All participants within the cohort were adults and provided hand-wipe samples in order to establish potential dermal exposure of phthalates. Personal and stationary air samples (24h) were collected and analyzed to estimate the human exposure through inhalation. Oral exposure via ingestion of food was assessed by taking liquid and solid food samples from a 24h duplicate diet. Dust samples from 61 homes were collected. All air bags were analyzed to monitor the exposure through skin contact, dust ingestion, ophthalmic and pulmonaty phthalate exposure. Analyses were carried out on a gas chromatography/triple quadrupole mass spectrometry (GC/MS/MS) in multiple reaction monitoring (MRM) mode. Validated extraction methods for selective and sensitive determination of phthalates esters, minimizing the sample cross-contamination will be presented, together with the measured concentrations, as well as a comparison of the estimated body burdens of phthalate concentrations with defined acceptable daily intake values (TDI) for children and adults. This research is part of the ‘Advanced Tools for Exposure Assessment and Biomonitoring’ (A-TEAM) project within a Marie Curie Initial Training Network.

MO275

PAH in settled house dust in selected European countries

Y. Mg, School of Geography Earth Environmental Sciences; S. J. Harrad, Polycyclic aromatic hydrocarbons (PAH), a group of pollutants with reported adverse health impacts including cancer, are emitted from a wide range of combustion activities in indoor environments (e.g. cooking, heating, etc.). While human exposure to PAHs via the diet and inhalation of indoor air has been widely studied, very little is known about exposure via contact with indoor dust present on surfaces such as floors, soft furnishings and tables etc. Thus, our study (by investigating around 400 samples from 40 UK homes plus 20 homes each from Finland, Czech Republic, Spain, and Greece) provides a valuable baseline evaluation of the range of exposure of European citizens to PAH via ingestion of indoor dust. We also examined potential putative sources of contamination with PAH in indoor dust. Our findings improve understanding of the PAH sources in settled house dust and inform strategies to reduce such contamination.

MO276

Dynamics of emerging and legacy contaminants in a tidal urban river

L. R. Holmhan, University of Rhode Island / Graduate School of Oceanography; M. C. Kaigh, U. R. M. Weinstein, New Jersey Institute of Technology

The transfer of hydrophobic organic contaminants (HOCs) from sediments into aquatic biota is of global concern especially where human activities have resulted in highly elevated concentrations of pollutants in the ecosystem. We investigated the trophic transfer and biomagnification of HOCs in the oligohaline-fresh portion of the tidal Passaic River estuary, a tributary of the Hudson River Harbor complex, extending from the Dundee Dam (river km 29) to its entrance at Newark Bay, New Jersey, USA (river km 0). We deployed passive samplers in the overlying water and used them to derive porewater concentrations and profiles of sedimentary HOCs. The following compound groups were targeted: polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorinated dibenzo-p-dioxins (PCDD/Fs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/DFs), as well as the currently used pyrethroids. Results for PAHs, PCBs and PCDD/Fs were recently published (Kaigh et al., 2014). For PCDD/Fs, only five PCDF congeners (BDE-37, BDE-47, BDE-99, BDE-100, BDE-189) showed a significant biomagnification along the food web. For these congeners, concentrations significantly increased with the increase in the trophic level (p < 0.05). Trophic magnification factors (TMFs) for the congeners with statistical significant relationship ranged from 2 (BDE-153) to 3.30 (BDE-47). For all the other congeners, the relation was statistically insignificant (p > 0.05), and the TMF ranged from 1 (BDE-15 to 2 (BDE-99).

MO277

Accumulation of the polyfluorinated sulfonate F53-B in food items from two provinces in China

R. Vesterbro, Stockholm University / Analytical Environmental Chemistry; H. Zhang, Research Center for Eco-Environmental Sciences Chinese Academy of Sciences; W. T. Wang, MTM Research Center; D. Herzke, NILU - Norwegian Institute for Air Research / MILK; G. Ji, Jiang, Perfluorokyl substances (PFASs) have received considerable attention from scientists and regulators over the past decade due to their persistence, bioaccumulation potential and toxicity. The concerns regarding the environmental and human health effects related to PFASs have initiated a transition to replace long-chain PFASs with short-chain alternatives and replacements with other bonds in the perfluorokyl chain. The rationale for favoring these replacements is based on the decreasing bioaccumulation factor or reduced environmental persistence. Yet, our understanding of PFAS replacements remain rather limited due to the lack of data. In this study, we analyzed market food items from the Hubei province (n=124) and the Zhejiang province (n=104) for the polyfluorinated ether sulfonate F53-B, which has been used as replacement for perfluorooctanoic sulfonic acid (PFOS) in China. An analytical method employing HPLC-MS/MS was developed to quantify F53-B in food items that previously have been analyzed for perfluorokyl acids. Overall, F53-B displayed a similar distribution as PFOS with the highest concentrations being observed in food samples of animal origin and particularly in liver samples from pig and duck (ranging up to 2.3 ng/g w/w). Collectively, these findings corroborate in silico estimations suggesting that F53-B is a highly persistent and bioaccumulative substance and underscores the need for human biomonitoring studies.

MO278

Very low levels of POPs in tern eggs from a remote island in the Indian Ocean

H. Bowman, North-West University / Environmental Sciences and Development; R. Chongw Kwet Iuye, University of Mauritius / Chemistry; H. Kylin, Linköping University / Department of Thematic Studies Environmental Change; A. Polder, The Norwegian School of Veterinary Sciences

The Indian Ocean is the world’s third largest and relatively unknown regarding pollution. In this study, we investigated POPs in tern eggs from Rodrigues 450 km east of Mauritius, an island populated by about 35 000 people. The levels were very low. We have now analysed eggs from terns on another Mascarene island, St Brandon’s Rock (SBR), located 400 km north of Mauritius, and with a transient population of about 25 fishermen. Ten eggs each of the Fairy Tern (FT, Gygis alba, also known as White Tern), Sooty Tern (ST, Sterna fuscata), and the Common Noddy (CN, Anous stolidus) were collected from SBR. Here, we report on the initial analyses of four eggs from each species in ng/g wet mass (wn). FT eggs were not collected on Rodrigues, as they occurred in low numbers. Mean ∑PCBs was 1.0 (FT), 1.1 (ST) and 1.0 (NC) ng/g wn. This is half the concentrations measured in ST and CN from Rodrigues (2.6 and 2.2 ng/g wn) respectively. For ∑DDT, the egg concentrations were 0.75 (FT), 0.95 (ST), and 1.0 (CN) ng/g wn, compared with 3.1 (ST) and 1.9 (CN) ng/g wn from Rodrigues, also half. The concentrations for HCB were 0.75 (FT), 0.33 (ST) and 0.52 (CN) ng/g wn, compared to slightly lower concentrations in eggs from Rodrigues; 0.41 (ST) and 0.39 (CN) ng/g wn, respectively. Mirex concentrations were 0.73 (FT), 0.43 (ST), and 0.65 (CN) ng/g wn in eggs from SBR, while ST and CN from Rodrigues had 0.69 and 0.77 respectively. For chlorodanes, chloronaphthalenes and BFRs, the concentrations were also low, and comparable between the tern species and the islands, with Rodrigues eggs slightly higher in most respects. One ST egg from SBR had HCB of 0.61 ng/g wn (the other eggs were below detection limit), possibly indicating ingestion of plastic. Provisionally, it can be concluded that although there were minor differences in concentrations between the species, the low numbers of eggs from only one species and one island will provide enough information for regular monitoring in this region. It should be kept in mind that even though the contaminant levels were in all respects low, industrialisation, conflict, development on the periphery, commercial exploitation of the coasts and oceans, and long-range transport of pollutants, including facility by marine debris, is likely to add to chemical pressure in this region. Monitoring the changes in contamination from a background site will be informative.

MO284

Accumulation of the polyfluorinated sulfonate F53-B in food items from two provinces in China
However, significant uncertainties in these global emission scenarios still persist. Simulations are conducted for the period 1930 baseline scenario (no export). To accomplish this task, we use the BETR Global 2.0 contri global fate and transport of PCBs using emission scenarios that include potential importers of wastes are expected. The second goal of this study is to simulate the scenario (i.e. no exports) and (ii) different long significantly increase as result of exports of wastes in comparison to reference emissions caused by the global flows of relevant wastes. In this study, we have therefore updated an existing global historical PCB emission inventory to account here might be a potential shift in scale. Over the last decade, a number of industrialized and developing nations. The potential emission of both emerging and legacy contaminants present in WEEE (e.g., organic chemicals, metals) during handling and disposal of these products is a concern with impacts at local to global scale. Over the last decade, a number of studies have reported surprisingly high levels of polychlorinated biphenyls (PCBs) within or outside developing regions in Asia and West Africa. As PCBs were never extensively produced nor used in these regions, this previously led us to hypothesize that there might be a potential shift in global source regions, attributed to exports of wastes containing PCBs towards developing regions. If true, it follows that global emissions, transport and exposures can neither be completely estimated, nor fully rationalized, without considering emissions caused by the global flows of relevant wastes. In this study, we have therefore updated an existing global historical PCB emission inventory to account for exports of wastes, using global flows of e-waste as a proxy for wastes containing PCBs. Our revised emission scenarios indicate that (i) global emissions may significantly increase as result of exports of wastes in comparison to reference scenario (i.e. no exports) and (ii) different long-term temporal trends in emissions with current production and use regions versus developing regions implicated as importers of wastes are expected. The second goal of this study is to simulate the global fate and transport of PCBs using emission scenarios that include potential contributions linked to the export of e-waste and compare model output to the baseline scenario (no export). To accomplish this task, we use the BErT Global 2.0 model, a publicly available fate/transport model, which represents the globe as 288 interconnected, multi-compartmentation zones at a spatial resolution of 15 x 19 degrees. Simulations are conducted for the period 1930–2100 and output from various locations and time periods are interpreted in the context of available monitoring data. Model output is also used to assess the potential implications of transboundary movement of wastes containing PCBs for human exposure in developing regions. However, significant uncertainties in these global emission scenarios still persist.

MO282 Polychlorinated biphenyls (PCBs) in transformer oils: Environmental Risk Assessment Challenges in Colombia

W. Ocampo-Duque, R. Sarria, Pontificia Universidad Javeriana Cali / Industrial Engineering; M. Schulmacher. Rovira i Virgili University / Chemical Engineering Polychlorinated biphenyls (PCBs) are widely used in a variety of applications mainly including dielectric fluids in transformers. These substances have been recognized by the scientific community as a threat to the environment and human health. In Colombia, according to very recent environmental regulations these substances must be eliminated from the electrical sector. Consequently, a project funded by UNEP is being carried to promote best technologies in the country for PCBs management. Therefore, some environmental research laboratories, as ours, are developing analytical methods for PCBs detection in diverse local environmental matrices. The final aim is to study the human health and environmental risks posed by these substances in the Colombian environment. PCBs were analyzed by GC/ECD from samples of transformer oils collected in cities from Colombia. A chromatograph Shimadzu GC-2010 Plus, donated by Spanish Government, equipped with a 68Ni ECD was used for analytical measurements. A column HP-5MS was used and samples were injected in split mode. Injection and ECD temperatures were set to 250 and 320 °C. The oven temperatures were conveniently programmed. Ar-CH4 was used as carrier gas at 1.23 mL/min. Calibration curves from 0.01 to 1 ppm were built to mix of Aroclors. The validation method was carried out following ISO 17025 methodology for QA/QC and Accreditation purposes. Practical quantification limits, and mean recoveries (>66%) were set up. Specificity, linearity, accuracy, repeatability and precision were good evaluated to the method. The analytical results showed that PCBs in the transformer oils may be identified for Aroclors 1242, 1245 and 1260. Real samples were analyzed and PCBs concentrations in transformer oils were between not detected and 328.25 ppm. PCBs concentrations of some samples exceed the 50 ppm national accepted limit. The analytical survey results suggested the presence of PCBs in the Colombian environment, and further research is required to estimate environmental risks due to the presence of such pollutants in Colombian cities.

Aquatic Nanotoxicology: from Freshwater to Seawater - overcoming the difficulties of standard toxicity tests and the implications for higher tier toxicity testing (P)

MO283 Size-dependent toxicity of silver nanospheres and nanowires to Glyptotendipes tokunagai

J. Jung, Korea University / Environmental Science and Ecological Engineering; S. Choi; T. Shim, Korea University / Environmental Science and Ecological Engineering; J. Park, Korea Institute of Toxicology / Center for Environmental Biotechnology Toxicity of silver nanomaterials (Ag NMs) is largely influenced by coating material, surface charge, size and shape. This study comparatively evaluated physicochemical properties of spherical silver nanoparticles (Ag NPs, diameter = 50, 100, 150 nm) and cylindrical silver nanowires (Ag NWs; length = 10, 20 μm) coated with polyvinylpyrrolidone (PVP) and their acute toxicity and bioconcentration to Glyptotendipes tokunagai. The aggregate and dissolution of the Ag NMs increased with increasing exposure time, which was more significant for Ag NWs compared with Ag NPs. In all experimental conditions, however, the dissolved concentrations of Ag ions from Ag NMs was lower than LC50 value for AgNO3 (3.51 mg L−1). This indicates that the toxicity of Ag NMs to G. tokunagai does not result from dissolved ions. Acute toxicity (48 h) of Ag NPs was the highest for the smallest particles (50 μm Ag NPs), whereas bioaccumulation (48 h) was the highest in the largest particles (150 μm Ag NPs). According to the result of bioaccumulation of Ag NPs as a function of time, bigger particles were found to be more quickly excreted. This indicates that smaller Ag NPs stay longer in the body of G. tokunagai, resulting in higher toxicity than larger Ag NPs. On the other hand, both acute toxicity and bioaccumulation of Ag NWs appeared higher with increasing length. The uptake of Ag NWs continuously increased within 48 h and the longer Ag NWs were absorbed more rapidly. In addition, acute toxicity of Ag NWs was compared with Ag NPs. In all experimental conditions, however, the dissolved concentrations of Ag ions from Ag NWs was lower than LC50 value for AgNO3 (101.81 mg L−1). This study suggests that the shape and size of Ag NMs have a significant effect on their acute toxicity to G. tokunagai. Considering that G. tokunagai, deposit feeder, recognizes larger particles as food more easily and eats them, this should be further studied relating to bioaccumulation and toxicity of Ag NMs.

MO284 Does Silver Nanocolloidal Disturb Medaka’s Defense to Pathogenic Bacteria? C. Kataoka, Toyo University / Life Sciences; S. Izumi, Tokai University; M. Fujita, Toyo University; S. Kashwada, Toyo University / Department of Life Sciences Size nanocolloids including silver colloid (SNC) have been mainly as antibacterial products. Ecological risks posed by silver nanomaterials has been internationally concerned because the amount of production and usage of nanomaterials is increasing. However, there are little information of the specific toxic mechanisms. From microarray data using embryos and post-hatch larvae of medaka exposed to SNC, immune-relative genes were significantly responded. Hence, we have a hypothesis that disturbed immune responses may lead to reducing of biological defense to pathogenic bacteria in medaka. To investigate exposure

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effects of SNC on medaka immune system, medaka embryos (developmental stages 11, 21, and 30) were exposed to 0.05 mg/L of SNC until hatching. Post-hatched larvae (stage 40) were also exposed for 7 days. Exposed eggs and larvae were subjected to qRT-PCR analyses of immune relative genes, such as nuclear factor kappa B (NFKB) p105 and NFKB p100, and tumor necrosis factor alpha (TNFα). In case of exposure from stage 11, NFKB p105 was suppressed, NFKB p100 and TNFα were enhanced compared with control. In exposure from stage 21, the three gene expression levels were decreased in a similar fashion. From stage 30 and in, the three gene expressions were all suppressed. Eggs exposed from stage 21 were subjected to whole mount in situ hybridization on day 3. Enhanced NFKB p105, NFKB p100, and TNFα genes were detected in pectoral fins and head; in liver and intestinal tract; and in ducts of Cuvier and heart, respectively. On the other hand, suppressed gene expressions of NFKB p105 were measured in Activities of TLR5, a receptor responsible for the binding of the metabolite of NFKB p105, and was decreased slightly by SNC exposure. It is well known that toll-like receptors (TLRs) signalings regulate these three factors and TLRs detect pathogen-associated molecular pattern. These findings strongly suggest that SNC can disturb immune responses and damage biological defenses to pathogenic bacteria in medaka.

MO287

Determination of silver in hemolymph of the marine amphipod (Parhyale hawaiana) as a tool to understand silver nanoparticles diet exposure. M. Vannucci-Silva, UNICAMP; M.C. Artal, University of Sao Paulo - USP; Toxicology and Toxicology analysis; G.D. Pessoa, S. Oliveira, M.A. Arruda, S. Cadore, State University of Campinas - UNICAMP; G.D. Umbuzeiro, FACULTY OF TECHNOLOGY -UNICAMP / LEAL.

Silver has been largely used in the production of nanomaterials and its release into the environment is becoming an environmental concern. The evaluation of bottom feeding organisms, like the marine amphipod Parhyale hawaiana, diet will be the most important route of exposure. The aim of this work was to evaluate a method to determine the exposure of AgNP through feeding in P. hawaiana. We will provide food with AgNP for adult organisms (0.5-1 mm). Determination of the internal dose of total Ag in the organism, as an exposure measure, will be used and hemolymph analysis was chosen for this purpose. Preliminary experiments were performed, aiming to develop methods for total Ag determination in hemolymph of adult animals that were exposed to different concentrations of AgNO3 in seawater. Determination of hemolymph Ag was made by ICP-MS after an extraction step. The results also show that, after 72h exposure, Ag+ was the most toxic form for both populations, followed by AgNPs 23, AgNPs 27 and AgNPs 20. No mortality was recorded for animals exposed to AgNPs 200. G2 appeared to be the most sensitive population and was selected for the second experiment. Thus, G2 individuals were exposed during 72h to 1 and 3 µg L-1 of Ag+, AgNPs 20, AgNPs 27 and AgNPs 20. The selected concentrations represented the EC5 and EC20 of AgNPs 23. Effects on survival, osmoregulation, antioxidant responses, defence mechanisms, cellular damage, energy reserves and behaviour were investigated. Ag+ and AgNPs 23 led to a significant decrease in survival rates, osmoregulation and locomotor activity. The other treatments resulted in no impact on survival and no impact on any of the studied responses. Soluble Ag from AgNPs in exposure medium were assessed using ICP-MS. The amount of soluble Ag was under the limit of quantification. The results showed that Ag+ and AgNPs 23 altered osmoregulation which appeared to be an effective marker as it indicates the physiological health status of G. fossarum. Locomotor activity, which was the most impacted response, reflects the potential effects of AgNPs 23 at the population level. In addition to highlighting the potential of G. fossarum as a model organism in nanotoxicology, this study illustrates that Ag+ and AgNPs 23 act at very low concentrations and their toxicity is highly dependent on their physico-chemical properties. Indeed, in addition to the size, this study demonstrated the importance of the methods of synthesis in nano-ecotoxicology.

MO288


Silver nanoparticles are frequently used in many consumer products because of their antibacterial and antifungal properties. The inevitable release of silver nanoparticles in the environment is becoming an environmental concern. Planktonic communities are of great environmental importance because they produce and transfer energy in the food web and they play a key role in nutrient recycling (e.g. via grazing and release of organic material). Planktonic organisms such as bacteria interact strongly with their ambient environment and are expected to be affected by exposure to silver nanoparticles, thereby potentially constituting the most endangered organisms in the aquatic environment. Silver nanoparticles may cause toxicity via multiple mechanisms such as inhibition of respiratory enzymes and induction of oxidative stress upon generation of reactive oxygen species (ROS) as well as inhibition of photosynthesis due to the accumulation of nanoparticles on the surface of photosynthetic organisms. The behavior of silver nanoparticles in the environment is complex and can differ from the respective behavior under standardized laboratory conditions, in terms of both abiotic and biotic factors and interactions with the surrounding environment. To date, most of the eco-toxicity studies with silver nanoparticles have been carried out mainly in freshwater systems, at much higher concentrations and over much shorter time periods than...
would ever be expected to occur in the future in a natural aquatic ecosystem. In this study, microcosm and mesocosm nanotoxicology studies in the marine environment were conducted focusing on realistic exposure conditions in the ng/L range. An aqueous suspension of branched poly(ethyleneimine) capped silver nanoparticles (AgNPs) of 60 nm size was used for this AgNP exposure study. Preliminary analysis revealed that, in the presence of silver nanoparticles, there was a decrease in the enzyme antioxidant activity, photosynthetic activity, chlorophyll concentration, and an increase in intracellular ROS formation and lipid peroxidation in planktonic communities which indicate that the organisms possibly underwent oxidative stress.

MO289
Size of agglomerates like a major contribution to silver nanoparticles aquatic toxicity
J. Oโปรs, University of Pardubice / Institute of Environmental and Chemical Engineering; M. Pouzar, University of Pardubice / Environmental and Chemical Engineering; P. Knutek, University of Pardubice; L. Blaha, Masaryk University, Faculty of Science / Faculty of Science RECETOX; P. Plodikova, Research Institute of Organic Synthesis
One of the general problems in aquatic toxicity testing of nanoparticles is the fast agglomeration and subsequent sedimentation, which limits the use of "standardized" protocols in the assessment of particle toxicity. The present work suggests an original approach enabling to control the maximal average size of nanoparticle agglomerates during the aquatic toxicity test. The modification is having different visible frequency of exposure vessel exchange, i.e. the frequency exchange of the tested vessel depending on the nanoparticle concentration levels. The method was applied for the short-term toxicity test with the embryo and sac-fry stages of common carp (Cyprinus carpio). Toxicity of silver nanoparticles (AgNPs) with initial average hydrodynamic diameter (Dh) of 40 nm was tested at four concentrations (5, 10, 25 and 50 nM). Based on the chemistry and behavior of AgNPs in the culture medium, two levels of maximal average sizes of agglomerates (200 and 400 nm) were selected, and exchanges of media were employed to control for formation of these types of agglomerates. The comparisons with the semi-static fishembryotoxicity test, where the formation of agglomerates had not been controlled, indicate that different sizes of agglomerates influence different stages of embryo development. Interestingly, larger agglomerates rather than original nanoparticle colloids were responsible for the most severe effects, which could be mechanistically explained by other observations during the study. Details on the novel testing approach and its advantages and limitations will be discussed.

MO290
Effect of silver nanowires on embryonic development of Daphnia galeata C. Rongxue, Konkuk University; Y. An, Konkuk University / Department of Environmental Science
Silver nanomaterials were widely used in various nanoproducts. This study investigated the effect of silver nanowires (AgNWs) using water flea Daphnia galeata embroyo. Two types of AgNWs tested were 10 um and 20 um AgNWs with different concentrations. Interestingly, larger agglomerates rather than original nanowires colloids were responsible for the most severe effects, which could be mechanistically explained by other observations during the study. Details on the novel testing approach and its advantages and limitations will be discussed.

MO291
Responses of antioxidant enzymes to nanoparticle and ionic silver in aquatic microorganisms
D. Barros, Universidade do Minho / Centre of Molecular and Environmental Biology Department of Biology; A. Pradhan, University of Minho / Department of Biology; P.M. Santos, Universidade do Minho / Centre of Molecular and Environmental Biology CBMA Department of Biology; C. Pascoal, F. Cassio, University of Minho / Centre of Molecular and Environmental Biology (CBMA); Department of Biology
Silver nanoparticles (AgNPs) are among the most used nanoparticles and are likely to be released in significant amounts to aquatic environments. Due to their antimicrobial properties, it is relevant to examine whether AgNPs can pose a risk to aquatic microorganisms in natural ecosystems. Here, we investigated the effects of AgNPs (citrate coated; 20 nm) on the growth and activity of antioxidant enzymes in i) two aquatic fangal strains of Articulospora tetradia, one isolated from a non-polluted stream (AT72) and the other from a metal-polluted stream (AT61), and ii) the bacterial strain Pseudomonas sp. M1 (PsM1) isolated from a sediment sample in a metal-polluted stream. The activities of glutathione peroxidase (Gpx), glutathione S-transferase (GST) and superoxide dismutase (SOD) were analyzed at AgNP concentrations inhibiting biomass production in 10% (EC50) and 20% (EC20) and the results were compared with those of ionic precursor (Ag+ in AgNO3). AT72 was the most sensitive strain to AgNPs, whereas PsM1 was the most tolerant one (EC20, 7.5, 158.9 and 300 µg L-1 for AT72, AT61 and PsM1, respectively). The activity of all tested enzymes increased after exposure to AgNPs and Ag+ in all microorganisms. However, at similar stress level, the increase in all enzyme activities was higher in AT72 than in AT61 indicating higher oxidative stress in the strain from the non-polluted stream. The exposure to both stressors led to higher stimulation of GPx and SOD in PsM1, whereas SOD and GST responses were more pronounced in fungi. This suggests that the enzymatic defense mechanisms against both forms of silver might differ between bacteria and fungi. The FEDER-POFC COMPETE and the Portuguese Foundation for Science and Technology supported this work (PIDDAC-Pest-OE/BAU/14050/2014, PTDC/AAC-AMB/12165/2010) and D. Barros (SFRH/BD/80407/2011).

MO292
Impacts of environmentally realistic silver nanoparticle exposure on Gammarus roeseli (Crustacea Amphipoda) from individual to functional levels.
J. Andreï, CNRS UMR 7360 / LieCNRS UMR; J. baquerel, Université de Lorraine; S. Paim-Devlin, Université de Lorraine - UL / LieCNRS UMR; L. Giambérini, Université de Lorraine, CNRS UMR 7360 / LieCNRS UMR; F. Guerold, Université de Lorraine / Laboratoire Interdisciplinaire des Environnements Continuits & PERSIST
By means of some antibacterial properties, silver nanoparticles (nAg) are used in current consumer products and released into aquatic environment. One of the main priorities is now to address an appropriate risk assessment by using environmentally realistic exposure concentrations and media. Because they are good integrators of environmental perturbations, the gammarids are currently used as model organisms to examine in vivo effects of nanoparticles functioning by their capacity to breakdown leaf-litter and release fine particulate organic matter (FPOM) in the aquatic ecosystem. The objective of this study was to evaluate the impact of low concentrations of nAg in gammarids on both the locomotion behavior and the respiratory response. Ventilation of gills provides oxygen for cellular bioenergetics ensuring sufficient ATP levels to perform the locomotion behavior. On the opposite, the locomotion behavior is an ATP-dependent process that may be primarily impaired when energy lacks. Thus monitoring these parameters could provide integrative information about nanoparticles effects. In this context, the effects of low concentrations (0.5, 2.5 and 5µg L-1) of nAg of different sizes (10, 20, 40, 60 and 100nm in diameter) with the same shape and coating were assessed on the G. roeseli species after 36 and 72h of exposure. The locomotor activity was monitored in a group of 8 gammarids. After that, we measured individually and on the same organism the nAg impacts on: the oxygen consumption, the ventilatory activity and the electron transport system (ETS). Concurrently, measures at the functional level such as the organism’s consumption and the FPOM production were performed. Realistic nAg concentrations did not induce mortality of gammarids despite a metal bioaccumulation. The first results showed an impact of the nAg on the gammarid oxygen consumption dependent of the nanoparticle size and concentration. The exposure to decreasing nAg sizes induced a proportional increase of the oxygen consumption by gammarids. For the 10nm nAg contamination condition, the highest concentrations induced also an increase of gammarid respiration. For 2.5um nAg contamination condition, the oxygen consumption by gammarids was 10% lower than controls. In the opposite, the locomotion behavior was an ATP-dependent process that may be primarily impaired when energy lacks. Thus monitoring these parameters could provide integrative information about nanoparticles effects. G. roeseli could affect the aquatic ecosystem functioning.

MO293
The influence of pH and media composition on suspension stability of Ag, ZnO, and TiO2 nanoparticles and immobilization of Daphnia magna under guideline testing conditions.
D. Cupi, DTU (Technical University of Denmark) / Department of Environmental Engineering; N.B. Hartmann, Technical University of Denmark (DTU) / DTU Environment; A. Baun, DTU Environment / Department of Environmental Engineering
In aquatic toxicity testing of engineered nanoparticles (ENPs) the process of agglomeration is very important as it may alter the bioavailability of the ENPs and hence their toxicity. In this study we evaluated test conditions that are more favorable in maintaining a stable and low agglomerate size profile of ENPs in aquatic media applicable in OECD guideline testing. Two media were used to investigate the effect of pH on suspensions stability (as point of zero charge (pzc) of the media) and the media composition. The results showed that a decrease in the EMN concentration led to a decrease in the agglomerate size. However, a dependence of the nanoparticles size and concentration on the organism’s respiratory behavior. These impacts are dependent of the nanoparticle size and concentration. Some potential effects on the energy metabolism of G. roeseli could affect the aquatic ecosystem functioning.
MO294
Multi Xenobiotic Resistance (MXR) vs Nanoparticles: is MXR a good biomarker of exposure? A study with zebra mussels and gammarids exposed to silver and titanium dioxide nanoparticles

M. Potet, J. Andrei, CNRS UMR 7360 / LIEC CNRS UMR; B. Marchal, Universite de Lorraine; M. Garaud, Laboratory LIEC - CNRS UMR 7360 - Udl / CNRS UMR 7360 - LIEC - Universite de Lorraine - CNRS / LIEC CNRS UMR; V. Felen, LIEC; L. Giamberini, Universite de Lorraine, CNRS UMR 7360 / LIEC CNRS UMR; F. Guerold, Universite de Lorraine / Laboratoire Interdisciplinaire des Environnements Continentaux; S. Pain-Devin, Universite de Lorraine - UL / LIEC CNRS UMR

Multi Xenobiotic Resistance (MXR) is a membrane efflux system involving ABC-transporters. Because MXR limits the cellular accumulation of a wide diversity of chemicals including environmental pollutants, it was shown to be protective against chemical injuries and was thus proposed to be used as a biomarker for the assessment of organism health. Numerous studies have shown that MXR is an effective defense against organic and metallic contaminants and it could also be involved in protection against nanoparticles. Since it becomes critical to worry about their environmental release and their potential effects on biota, we wonder whether MXR could reveal the exposure of organisms to this new class of environmental pollutants. The present study addresses this question by monitoring the response of MXR in two freshwater invertebrates from different habitats, trophic levels and functional roles. While zebra mussels (Dreissena polymorpha) is a filter-feeding bivalve responsible for clearing waters and for organic matter degradation, gammarids are bottom feeders responsible for the flux and redistribution of matter and energy. The poster presents an overview of the results from both short-term (72h in microcosm) and long-term exposures (up to 21 days in mesocosm) of mussels and gammarids to different types and concentrations of silver (PVP-coated plates 35μm, citrate coated spheres 10, 20 and 60μm – from 0.5μg/L to 90μg/L) and titanium dioxide (Rod-shaped and cubic anatase, rutile – from 20μg/L to 1mg/L) nanoparticles (NP). MXR was assessed by measuring the accumulation of fluorescent dye in gill tissues with or without inhibitors of efflux transporters. The Cohen d effect-size between inhibited and non inhibited conditions gives a picture of MXR activity. The results showed differential responses of MXR depending on the invertebrate species, the NP type, the exposure concentration and time. Since different responses were observed in mussels and gammarids, concentrations as low as 0.5μg/L caused the most pronounced response when compared to higher concentrations and to toxic silver. Titanium dioxide NP caused high, early and transient inductions of MXR especially at lower concentrations. Though there is a lack of knowledge on the mode of interaction of NP with MXR, this biomarker seems to be a promising tool for NP exposure assessment.

MO295
Effect of salinity and DOC gradients on the stability of TiO2 nanoparticle aggregation and their effects towards plankton from Lagoon of Venice (Italy)

V.I. Slavuykova, University of Geneva / Section of Earth and Environmental Sciences Institute Foroletti and Institute for Environmental Sciences; C. Pestrinuia, University of Geneva / Institute Foroletti Earth and Environmental Sciences; S. Le Faucheur, Section of Earth and Environmental Sciences Institute Foroletti and Institute for Environmental Sciences; M. Mortimer, Bren School of Environmental Science and Management; S. Stoll, University of Geneva / Institute Foroletti Earth and Environmental Sciences; F.B. Aubry, ISMARCNR; N. Bott, RMIT University / Biotechnology and Environmental Biology; R. Zonta, ISMARCNR

Understanding of the fate and effects of engineered nanomaterials in highly dynamic natural environment is a precondition for reliable assessment of their risks. However there is a paucity of studies exploring their behaviour and effect in natural transitional environments. Therefore salinity and dissolved organic matter (DOC) concentrations gradients on the nano TiO2 limits the cellular accumulation of a wide diversity of chemicals including environmental pollutants, it was shown to be protective against chemical injuries and was thus proposed to be used as a biomarker for the assessment of organism health. Numerous studies have shown that MXR is an effective defense against organic and metallic contaminants and it could also be involved in protection against nanoparticles. Since it becomes critical to worry about their environmental release and their potential effects on biota, we wonder whether MXR could reveal the exposure of organisms to this new class of environmental pollutants. The present study addresses this question by monitoring the response of MXR in two freshwater invertebrates from different habitats, trophic levels and functional roles. While zebra mussels (Dreissena polymorpha) is a filter-feeding bivalve responsible for clearing waters and for organic matter degradation, gammarids are bottom feeders responsible for the flux and redistribution of matter and energy. The poster presents an overview of the results from both short-term (72h in microcosm) and long-term exposures (up to 21 days in mesocosm) of mussels and gammarids to different types and concentrations of silver (PVP-coated plates 35μm, citrate coated spheres 10, 20 and 60μm – from 0.5μg/L to 90μg/L) and titanium dioxide (Rod-shaped and cubic anatase, rutile – from 20μg/L to 1mg/L) nanoparticles (NP). MXR was assessed by measuring the accumulation of fluorescent dye in gill tissues with or without inhibitors of efflux transporters. The Cohen d effect-size between inhibited and non inhibited conditions gives a picture of MXR activity. The results showed differential responses of MXR depending on the invertebrate species, the NP type, the exposure concentration and time. Since different responses were observed in mussels and gammarids, concentrations as low as 0.5μg/L caused the most pronounced response when compared to higher concentrations and to toxic silver. Titanium dioxide NP caused high, early and transient inductions of MXR especially at lower concentrations. Though there is a lack of knowledge on the mode of interaction of NP with MXR, this biomarker seems to be a promising tool for NP exposure assessment.

MO296
The Effect of Different Dispersion Methods on TiO2 Nanoparticle Ecotoxicity

A. Erdem, Akdeniz University / Department of Environmental Engineering; M. Ozkalleli, Akdeniz University / Dept. of environmental engineering

In recent years, the rapid development of nanotechnological activities, nanoparticles (NPs) emerge in many commercial products, from paints to cosmetic creams and from textile products to children toys. The engineered NPs need to be evaluated in a broad biosafety assessment studies. NPs have unique physical and chemical properties, which would be an important factor in determining the possible effects on ecosystem health and the prevention of problems before they occur. However, there are limited scientific studies on the effects of NPs, and there is no environmental standard and implementations on the NPs, yet. The main mechanism of the inactivation of algae, the roles of TiO2 NPs in the inactivation of those organisms, the properties of the NPs affecting the ecotoxicity still remain unknown. Moreover, there is a lack of standard methods on NP suspension preparation and NP characterization. Depend on the different environmental conditions applied in the experimental systems, NP size and size distributions are highly affected by the applied conditions. Especially prior to the experiments, both particle size and agglomeration state of the suspension strongly depend on the dispersion regime. When current legislation on NPs were evaluated, no standard method on NP suspension preparation could be found. EU commissions recommend for futher studies on standardization of those methods and conducting experiments to assess the NP behavior and fate of the NPs in real environmental conditions in technical reports. In this study, the effects of different concentrations (1, 10, 50, 100 mg/L) of TiO2 NPs will be prepared with different dispersion methods (mixing, probe sonication and bath sonication) in synthetic surface water (SSWs) samples with different water quality parameters (very soft to very hard, low to high alkalinity, pH 6.5 – 7.5) on green- algae P. subcapitata were evaluated. Algal inhibition, membrane deformation and cellular respiration inhibition analyses were conducted. The results were expected to confirm the influence of the dispersion procedure on the NP properties and consequently on the toxicity of the NPs. The procedures differed in terms of dispersion method, time, and media. The results show that the resulting NP size in different SSWS showed a strong dependence on the dispersion and the dilution method. Authors would like to acknowledge the financial support from TUBITAK (114D105), Turkey.

MO297
In vivo exposure of marine clams to titanium dioxide nanoparticles: responses in haemolymph, gills and digestive gland

L. Biagioli, University of Padua / Department of Biology; V. Matozzo, University of Padova / Department of Biology

The rapidly growing use of nanotechnology products in a variety of applications is responsible for increased discharge of nanoparticle (NPs) into costal marine environments. However, little is known about fate and potential toxicity of NPs in the aquatic biota. In this regard, efforts should be addressed to the evaluation of the biological impact of NPs in biologically and ecologically relevant species. Titanium dioxide (nTiO2) is one of the most common nanoparticles and is part of many commercial products such as sunscreens, cosmetics, and paints. During recent years several studies have been performed demonstrating that this NP can affect biological responses at different levels of organization. In this study, Radiapes philippinarum was selected to assess the potential adverse effects of nTiO2 under in vivo exposure. Clams were exposed for 7 days to environmentally realistic concentrations of nTiO2 (0, 1, 10 μg/L) and bulk TiO2 (10 μg/L). Bulk TiO2 was used to assess the potential differing action of metal oxide compared with the respective NP. At various time intervals during the exposure, several cellular and biochemical responses were evaluated in clam haemolymph, gills and digestive gland. NPs caused increases in glutathione and changes in haemocyte proliferation. A modulation of both haemocyte Neutral Red uptake and lysozyme activity in cell-free haemolymph was also observed in NP exposed clams. TiO2 NPs genotoxicity in haemocytes was revealed by the assay at both concentrations tested. DNA alterations may be due to ROS generation in cells, given that in gills and digestive gland an induction of antioxidant enzymes was found. Indeed, significant increases in catalase and superoxide dismutase activities, and changes in glutathione transferase activity, were showed. Only in digestive gland an increase in lipid peroxidation was revealed under nTiO2 exposure. Different responses in nTiO2- and bulk TiO2-exposed clams, the latter showing overall lower detrimental effects, suggested different mechanism of action, toxicity of nTiO2 depending not only on TiO2 molecules, but also on NP specific features.
Mussels were exposed to each form of zinc for 96 hours, then placed in air to dissolve zinc or nanozinc oxide and air. The results of this study show that ZnO nanoparticles are not acutely toxic to mussels exposed to the NPs. In the microcosm experiment there was 100% mortality after 7 weeks at 5 mg/L for dissolved zinc and was > 50 mg/L for nanozinc oxide based on shell opening responses. However, the air-time survival dropped from 19 days in control to 2 days in nanozinc oxide at 2.5 mg/L nanozinc oxide for 96 h. The air-time survival did not significantly change in mussels exposed to the same concentration of dissolved Zn. Significant weight losses were observed at 0.5 mg/L nanozinc oxide and at 2.5 mg/L for dissolved zinc chloride, and were also significantly correlated with air-time survival (r=0.53: p<0.01). Air exposure significantly increased COX activity in control mussels and in mussels exposed to 0.5 mg/L nanozinc oxide and zinc chloride. COX I and COX II activities were also enhanced after air exposure where both forms of zinc increased this effect. The data suggest that air-time survival could be affected by exposure to chemicals such as dissolved and nanozinc oxide. Exposure to air produced an inflammation reaction (COX activity) and weight loss in mussels, which are also influenced by the presence of metals and nanoparticles, further suggesting that health impacts of prolonged exposure to air from low water levels could be increased in the presence of environmental contaminants.

MO301
ZnO nanoparticles and bulk form and ionic Zn cause comparable toxicity in mussels, without evidence for Zn bioaccumulation
A.J. Romero, Zoology and Animal Cell Biology Research Centre for Experimental Marine Biology, PUCPV/UEHU; U. Izagirre, University of the Basque Country (UPV/EHU); M.P. Cajaraville, University of the Basque Country; I. Marigomez, Euskal Herriko Unibertsitatea / Zool Eta AnimZelulen DinamSaila; M. Soto, University of Basque Country / Zoology and Animal Cell Biology Research Centre for Experimental Marine Biology and Biotechnology PUCPV/UEHU
The aim of the present investigation was to understand bioaccumulation, cell and tissue distribution, and biological effects of ZnO nanoparticles (NPs) of two different sizes and shapes in marine mussels, Mytilus galloprovincialis. With that purpose, mussels were exposed in vivo to 0.07, 0.7 and 7 mg Zn/L either as ZnO NPs stabilized with Ecodis P90 of 20-70 and 500x260x10-100 nm (ZnO-NPs) or ZnO 130 to 200 μm and 50 g L-1 (bulk ZnO). A high mortality was recorded after exposure to all the Zn forms, except at 0.07 mg Zn/L. ZnO NPs exhibited a high solubility and agglomeration in seawater, but however Zn accumulation in mussel soft tissues was comparable to that found in control mussels, probably due to efficient mechanisms of metal handling developed by mussels. Nevertheless, severe histopathological alterations were observed in the digestive gland after exposure to all Zn forms, especially after ZnO NP exposure (i.e. epithelial thinning and vacuolization of the digestive cells, apoptotic nuclei in epithelial cells, disorganization of the interstitial connective tissue and haemocytic infiltration, necrosis of muscle tissue). Lysosomal membrane stability was reduced in mussels exposed to all forms of Zn even if intralysosomal metal accumulation could not be confirmed by autometallography and X-ray microanalysis. In conclusion, ZnO NPs behaved similarly to the bulk and ionic forms of Zn. The results indicated that exposure of nZVI to mussels could be affected by exposure to chemicals such as dissolved and nanozinc oxide. Exposure to air produced an inflammation reaction (COX activity) and weight loss in mussels, which are also influenced by the presence of metals and nanoparticles, further suggesting that health impacts of prolonged exposure to air from low water levels could be increased in the presence of environmental contaminants.
Effects of nFe0 on growth of the alga Pseudokirchneriella subcapitata

G. Libralato, Veneto Nanotech SCPa / Department of Environmental Sciences Informatics and Statistics; P. Rousseau, LIEC CNRS Université de Lorraine; B. Yang, LIEC CNRS Université de Lorraine; C. Reinhardt, Université de Lorraine; CNRS UMR 7360 / LIEC CNRS UMR; D.A. Vigna, CNRS UMR.

The high reactivity and catalytic properties of zero-valent iron (nFe0) are finding several applications in environmental remediation. Field concentrations during typical application can reach between 5 and 50 g/L. Investigations were carried out to evaluate the effects of four types of commercial engineered nFe0 along with bulk (FeCl3) and micro-sized Fe0 and the citrate coating present in just one nFe0 type. In this study, the short-term chronic toxicity (72 h) to Pseudokirchneriella subcapitata (Korschikov) was investigated in reconstructed freshwater according to ISO standard 8692 using a fluorimetric method. Iron nanoparticles ranged between 25 and 50 nm, whereas the batch of micro-sized ones was averagely of 60 μm (TEM). Suspensions of nanomaterials were prepared in ultra-pure water, sonicated, aged for at least 1 day, vortexed and transferred to the 96-well plates. Algae and growth medium were further added to reach the optimal initial exposure concentration according to the standard protocol. Experiments were iterated three times at in at least three replicates per treatment. Results allowed to rank Fe0 particles evidencing median inhibition concentration effects (IC50) of 18, 329, 512 and 967 mg/L. Bulk iron resulted more toxic, IC50=2 mg/L, than nano-sized particles, while micro-sized ones were not toxic within the tested range till 1600 mg/L.

Effect of nFe0 on egg hatching success of the green alga Chlamydomonas reinhardtii

K. Parramore, University of Eastern Finland / Department of Biology; G.C. Waiss-Leinonen, University of Eastern Finland; I. Nybom, Department of Biology; J. Akkanen, University of Eastern Finland / Department of Biology. Fullerenes (nC60) are used in commercial products in increasing volumes, which enhances their risk to be released into the environment. Some applications provide a disposal of aquatic endotoxins and other harmful biocides or systems designed to remove nanoparticles. Thus, aquatic species can be exposed to fullerene. Despite that C60 are stable molecules and can remain in water for months or years, only few studies have focused on their long term effects, and even less on effects through generations. This study presents effects of nC60 in lifetime-exposure on crustacean Daphnia magna through three generations. Each generation were exposed to equal nC60 concentration and their offspring were examined for higher accumulation of fullerene, and changes in survival, growth, offspring production, and histology.

Effects of copper oxide nanoparticles and light on the green alga Chlamydomonas reinhardtii

G. Cheloni, Institute Forel / Earth and Environmental Sciences; E. Marti, University of Geneva / Environmental Geochemistry Institute; F.-A. Forel, Earth and Environmental Sciences, Faculty of Sciences; V. I. Slaveykovsky, University of Geneva / Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences

Light is one of the most important and most variable factor in natural environments and it might represent an environmental stressor to aquatic organisms. Standard toxicity tests are performed under constant and optimal light conditions and the influence that light might have on pollutant speciation, bioavailability and toxicity are never taken into account. Many nanomaterials are produced and applied thanks to their optical and photocatalytic properties, however, excepted for TiO2 and ZnO nanoparticles (NPs), no or little information are available about their phototoxicity to aquatic organisms once released into the natural environment. With this work we explored the effects of visible and UV light to the stability of CuO NP dispersion and its toxicity to the green alga Chlamydomonas reinhardtii. C. reinhardtii was exposed to 0.8 mg/L CuO NPs under three different light conditions: 1) a low PAR laboratory condition, 2) a solar-simulated light (PAR+UV) and 3) a solar-simulated light (PAR+UV+B) with increased UVB radiation. Rates of growth inhibition and changes in chlorophyll a, Chl a/b, and cell division of ROS were measured via flow cytometry after 2, 4, 8 and 24 hours exposure. The evolution of nano CuO hydrodynamic size, zeta potential and dissolution through time was studied under the chosen light conditions. The chosen UVB light condition in the treatment PAR+UV+B resulted to be highly toxic for C. reinhardtii and in presence of CuO NPs the toxic effect was further increased. Synergistic effects were observed and induced in cell death by the combined exposure to light and intracellular ROS. The combined exposure of CuO NPs and solar simulated light (PAR+UV) had a synergistic effect on the induced oxidative stress. This finding contrast with our previous results obtained for combined exposure to light and ionic Cu(II), in that study antagonistic interactions were observed. The increase of intracellular ROS caused by CuO NPs in presence of visible light suggests that photocatalytic production of ROS might occur. The results of the present study imply that nanomaterial with specific optical properties might have increased toxicity to aquatic microorganisms under environmentally relevant light conditions. The introduction of light as variable factor in toxicity tests is highly sought to better estimate the potential risk correlated to the release of oxide nanoparticles into the aquatic environment.
generation to generation which could lead more effective transportation in the food web over the time. There was no effect on survival of individuals, but possible changes in growth and the offspring production in \textit{r}_{max} exposure compared to clean water controls were observed. Reduced offspring production may weaken population and thus reflect to upper levels of the foodweb.

**MO308**

**Investigations on the effect of wastewater bower contaminates on Hyalella azteca using two coupled test systems: a case study with nanomaterials**

C. Schlechtriem, Fraunhofer IME / Oekotoxikologie; K. Schlich, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; K. Hund-Rinke, Fraunhofer IME

Today we have a large variety of contaminants, which enter sewage treatment plants (STP) through the sewer system. For some of these substances, such as antibiotics it was shown that they leave the STP to a large extent within the effluent. For other substances, such as nanomaterials (NM) it was shown that they are efficiently adsorbed to sewage sludge and only a smaller part passes the STP. The fate and effect of NM in different matrices and on various organisms was already investigated under standardized laboratory conditions. Aquatic organisms are quite sensitive, if they are exposed to NMs. However, by passing STPs, NM are transformed to various degrees and their effect on aquatic organisms may differ from those obtained with standardized test systems. Therefore, there is a need to gather detailed information about the fate and behavior of NM under environmentally relevant conditions and their effect on aquatic organisms. Unfortunately, most test systems commonly used for aquatic ecotoxicity testing are not suitable to be used in studies investigating STP effluent. The main goal of this study was to implement a test system to investigate the implications of environmentally relevant conditions on (I) the fate of NM in a STP and (II) the resulting effects on aquatic organisms. To achieve our goal we used a combination of two test systems. A STP was simulated in accordance to OECD TG 303A with a continuous influent of various NM concentrations over 10 days. During the exposure period the fate of NM in the STP was assessed by chemical analysis. In the following the effect of NM in STP effluent on the amphipod \textit{Hyalella azteca} was investigated in a prolonged toxicity test. We found that \textit{H. azteca} is quite robust against the exposure to STP effluent. Due to this they present a suitable test organism for the purpose of our study. Testing NM under environmentally relevant conditions is an important point regarding the risk assessment of NM. In addition, with the suggested test system, the influence of various substances, which reach the STP and leave it via the effluent such as antibiotics or other drugs and their metabolites, on aquatic organisms can be examined.

**MO309**

**The role of feeding behaviour in the uptake of microplastics in two aquatic crustaceans: Gammarus locusta (Amphipoda) and Daphnia magna (Cladocera),**

R. Aljaibachi, University of Reading / Biological Science; A. Callaghan, K. Chappell, University of Reading

Unfortunately, most test systems commonly used for aquatic ecotoxicity testing are not suitable to be used in studies investigating STP effluent. The main goal of this study was to implement a test system to investigate the implications of environmentally relevant conditions on (I) the fate of NM in a STP and (II) the resulting effects on aquatic organisms. To achieve our goal we used a combination of two test systems. A STP was simulated in accordance to OECD TG 303A with a continuous influent of various NM concentrations over 10 days. During the exposure period the fate of NM in the STP was assessed by chemical analysis. In the following the effect of NM in STP effluent on the amphipod \textit{Hyalella azteca} was investigated in a prolonged toxicity test. We found that \textit{H. azteca} is quite robust against the exposure to STP effluent. Due to this they present a suitable test organism for the purpose of our study. Testing NM under environmentally relevant conditions is an important point regarding the risk assessment of NM. In addition, with the suggested test system, the influence of various substances, which reach the STP and leave it via the effluent such as antibiotics or other drugs and their metabolites, on aquatic organisms can be examined.

**MO310**

**Combined effects of gold nanoparticles, microparticles and temperature increase on the common goby (\textit{Pomatoschistus microps}): predatory performance, biomarkers and gold body burden**

P. Ferreira, ECT Oekotoxikologie GmbH; E. Fonte, ICBAS CIMAR University of Porto; M. Soares, F. Carvalho, UCBIO-REQUIMTE University of Porto; Laboratory of Toxicology Department of Biological Sciences Faculty of Pharmacy; L. Guilhermino, CIBAR University of Porto

The contamination of estuaries and other marine ecosystems by nanoparticles and microplastics (MP), and the effects of temperature variation on the toxicity of these substances raise a high concern in relation to their potential adverse effects on environmental and human health. Despite the research that has been done in the last years, considerable gaps of knowledge still exist, particularly on the combined effects induced by multi-stressors exposure. Thus, the effects of gold nanoparticles (AuNP) and MP on the common goby (\textit{Pomatoschistus microps}), a key-stone species for several European coastal ecosystems were studied. Short-term (96h) laboratory bioassays with early \textit{P. microps} juveniles exposed to AuNP and MP alone and in combination (ppb range) were carried out at both temperatures. Post-exposure predatory performance (PP), the activity of the enzymes acetycholinesterase (ACHE) and glutathione S-transf erase (GST), and lipid peroxidation levels (LPO) as effect criteria. The post-exposure gold content of fish was also determined. At 20°C, AuNP alone caused a significant reduction of the fish predatory performance; MP alone caused no significant effects; and the AuNP/MNP mixture caused a decrease of the predatory performance and increased LPO levels. The 5°C increase of temperature modified the effects induced by AuNP, MP and the mixture in some of the analysed parameters, and increased the concentration of gold in the body of fish exposed to AuNP alone and in combination with MP. Overall, the results indicate that AuNP (ppb range) are able to induce toxic effects on \textit{P. microps} juveniles, and that both MP and temperature influence the toxicity of these nanoparticles to the tested species. These findings highlight the need for more research on the combined effects of multi-stressors.

**MO311**

**Examination of nanoparticle uptake in Euglena gracilis**

X. Li, Environmental Toxicology; K. Schirmer, L. Sigg, Eawag / Environmental Toxicology; R. Behra, Eawag / Department of Environmental Toxicology

Worldwide, the use of engineered nanoparticles incorporated in consumer products has started to grow significantly. The entry of nanoparticles into aquatic environment is likely, with potential impacts on freshwater ecosystems. Among the few studies concerning interactions of nanoparticles with algae, toxic effects were documented and the effective concentrations were reported. While it has become clear that nanoparticles can be internalized into mammalian cells through plasma membrane, most likely via endocytotic mechanisms, little is known on the uptake of nanoparticles in algae. The presence of a cell wall in addition to the plasma membrane has been considered as an barrier against nanoparticle entrance. In the present study, we examined the uptake of fluorescent polystyrene nanoparticles (PSNP), 50 and 500 nm, in the alga \textit{Euglena gracilis}. Instead of a typical cell wall, this alga species has a pellicle in surrounding the plasma membrane, and the pellicle stripes are arranged diagonally on the surface with alternating pattern of ridges and grooves. For the experiment, algae were cultured phototrophically and heterotrophical and were exposed to 0.25 mg/mL PSNP in both culture medium under light at 20°C in a shaker. After 2, 24 and 72 hours, the cells and PSNP were imaged with confocal laser scanning microscopy. The confocal microscopy images did not evidence any uptake of PSNP in \textit{E. gracilis} cells after 2 h exposure. The 50 nm PSNP were found mostly aligned with the pellicle stripes while in case of 500 nm PSNP, no nanoparticles were found associated with the cells. Long-term exposures for 1-3 days lead to cell death. These results suggest that nanoparticle internalization in \textit{E. gracilis} cells is unlikely, even though cells are cultured in heterotrophic medium. The size preference for PSNP adsorption onto pellicle indicate that the adsorption is most likely driven by physical entrapment within the pellicle stripes. Key words: nanoparticle, algae, uptake, adsorption

**Biophysical Interactions at the Bio-nano Interface: Relevance for Aquatic Nanotoxicology (P)**

**MO312**

**Toxicity of CuO nanoparticles to freshwater algae**

J. Zhao; Z. Wang, Ocean University of China; B. Xing, Department of Plant, Soil and Insect Sciences / University of Massachusetts

CuO NPs exhibited toxicity to the eukaryotic alga \textit{Chlorella pyrenoidosa}. This alga had a higher tolerance for CuO NPs than the prokaryote alga, with a 72 h EC40 at 45.7 mg/L. Scanning electron microscopy (SEM) images showed that CuO NPs were attached on algal cells and interacted with extracellular polymeric substances (EPS) excreted by algae. Further transformation of CuO NPs was observed via TEM analysis. The toxicity of CuO NPs did not evidence any uptake of PSNP in \textit{E. gracilis} cells after 2 h exposure. The 50 nm PSNP were found mostly aligned with the pellicle stripes while in case of 500 nm PSNP, no nanoparticles were found associated with the cells. Long-term exposures for 1-3 days lead to cell death. These results suggest that nanoparticle internalization in \textit{E. gracilis} cells is unlikely, even though cells are cultured in heterotrophic medium. The size preference for PSNP adsorption onto pellicle indicate that the adsorption is most likely driven by physical entrapment within the pellicle stripes. Key words: nanoparticle, algae, uptake, adsorption

**MO313**

**A certain shade of green: new insights into shading effects of nanoparticles on fish predatory performance; MP alone caused no significant effects; and the AuNP/MNP mixture caused a decrease of the predatory performance and increased LPO levels. The 5°C increase of temperature modified the effects induced by AuNP, MP and the mixture in some of the analysed parameters, and increased the concentration of gold in the body of fish exposed to AuNP alone and in combination with MP. Overall, the results indicate that AuNP (ppb range) are able to induce toxic effects on \textit{P. microps} juveniles, and that both MP and temperature influence the toxicity of these nanoparticles to the tested species. These findings highlight the need for more research on the combined effects of multi-stressors.**

**MO314**

**A certain shade of green: new insights into shading effects of nanoparticles on**
algal growth
R. Hjorth, Department of Environmental Engineering; S.N. Sørensen, DTU Environment / Department of Environmental Engineering; M.E. Olsson, Technical University of Denmark / Dept of Environmental Engineering; N.B. Hartmann, Technical University of Denmark (DTU) / DTU Environment
Inhibitory effects of nanoparticles on algal growth have been the subject of many scientific studies, of which test designs are also required to elucidate the mode of action as a potential case of effects. Most of these studies have applied a physical separation of algal cells and nanoparticle suspensions (e.g. Hartmann et al., 2010). By using such methods, shading has generally been rejected as a cause of effects. For very dark suspensions of platinum nanoparticles tested in a double-vial setup, however, shading was found to contribute to the observed effects (Soerensen et al., 2012). Nevertheless, test designs cannot be causally related to a cellular level caused by the encapsulation of the cells by particles. Encapsulation of the algal cell may lead to a change in the physical growth conditions (light intensity). A change in chlorophyll/carotenoid pigment ratio upon nanoparticle exposure has been hypothesized to indicate shading on a cellular level (Wei et al., 2010). Pigment composition is known to be affected by changes in light conditions as a result of photoaclimation. Here we investigate the pigment composition of green algae, *Pseudokirchneriella subcapitata* and how the composition change as a result of nanoparticle exposure. Test glass vials with 4 mL algae and test solutions were incubated on a shaker (300 rpm) at 20 ± 2 degrees C and continuously illuminated using a cold light fluorescent tube emitting light in the visible spectrum. We made a light intensity ‘calibration curve’ using natural density filters that allow for 25%, 50%, 25%, 13%, and 6% light transmission respectively and analysed the pigment composition and content in the incubated algae by HPLC. Based on this calibration curve, comparisons were made to the pigment composition in algae exposed to nanomaterials. These investigations will assist to elucidate the mechanism effects of nanoparticles towards algae. Hartmann NB, et al., 2010, Toxicology 269(2-3):190–197. Soerensen SN, et al, 2014, in *Science Across Bridges, Borders and Boundaries*, Programme Book, SETAC-Europe, Basel, Switzerland, Wei, C. et al, 2010, Journal of Environmental Sciences 22(1): 155–160.

MO314 Comparative performance of three photosynthetic species exposed to an engineered nanoparticle with anti-corrosion properties
R. Martins, Department of Biology, University of Aveiro / Department of Biology and CESAM Centre for Environmental and Marine Studies; M. Avelelas, Smallmatek Small Materials and Technologies Lda; D. Tedim, Universidade de Aveiro / Department of Materials and Ceramic Engineering CICECO; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; S. Loureiro, Universidade de Aveiro / Department of Biology and CESAM; D. Tedim, Universidade de Aveiro / Biology
Corrosion of metallic structures is a serious worldwide problem, which has been minimised with environmentally harmful corrosion inhibitors that are added to protective coatings. Recently, some of those active compounds were successfully encapsulated in novel “smart” engineered nanomaterials, namely layered double hydroxides (LDHs). LDHs can be loaded with corrosion inhibitors such as 2-methylthioglycolic acid (MTA), storing and releasing MTA only in specific conditions coinciding with degradation phenomena. A potential advantage of this approach is the limitation of spontaneous leaching of the added active ingredients into the environment. In spite of LDHs being considered as prospective additives for protective coating systems, both active compounds and nanocantainers may also be bioavailable to organisms when released from the coating. Considering their potential to become environmentally relevant, the behavior and toxicity of zinc oxide and silver nanoparticles towards the green freshwater algae *Chlamydomonas reinhardtii* was investigated as part of the EU project NanoMILE. In a first step, the nanoparticles were characterized in an exposure medium with an ion composition similar to the surface waters of Switzerland. The changes in the mean particle size, polydispersity index, zeta potential and the amount of released metal ions were measured over time. To investigate, in the second step, the inhibition of the growth of the diatom *Phaeodactylum tricornutum* and the green alga *Tetraselmis chuii* that showed the highest toxicity to the diatom *P. tricornutum* unloaded ENMs did not induce toxicity in both species, apart from 100 mg Sln.L⁻¹ that showed a significant growth inhibition. The encapsulated biocides were less toxic for both species than the free ingredients. Further studies should be carried out to assess potential biochemical and physiological changes on non-target species, in order to have a holistic overview of their toxicity and to promote a safe design of those ENMs. Sources for differences in silver nanoparticle toxicity at standardized conditions can be numerous. They range from particle properties and their actual concentrations to sources for differences in silver nanoparticle toxicity at standardized conditions can be numerous. They range from particle properties and their actual concentrations to differences in particle and bioaccumulation of silver ions. However, as a function of the free silver ion concentration, silver nanoparticles were more toxic. Nevertheless, in the presence of cytoine, a strong ligand for silver ions, the adverse effects of silver nanoparticles were neutralized, indicating that silver nanoparticle toxicity is solely mediated by free silver ions. The interactions of silver ions with the cellular components of algae differ significantly from other nanoparticle species, as it influences the particle dissolution and bioaccumulation of silver ions. However, upon exposure to silver, algae cells incite a defense mechanism which enables them to mitigate the silver toxicity and to recover over time.

MO315 Influences of the coating on silver nanoparticle toxicity in a chronic test with *Daphnia magna*
Y. Sakka, University of Bremen / General and Theoretical Ecology; A. Mackeivica, Technical University of Denmark / Environmental Engineering; L.M. Skjolding, DTU / DTU Environment; A. Baum, DTU Environment / Department of Environmental Engineering; P. Heise, University of Bremen / Environmental Toxicology
Silver nanoparticles induced a 20 times lower toxicity (in terms of EC₅₀) on the growth rate, photosynthetic yield and the energetic state of the cell than silver nitrate, based on the total added silver. However, as a function of the free silver ion concentration, silver nanoparticles were more toxic. Nevertheless, in the presence of cytoine, a strong ligand for silver ions, the adverse effects of silver nanoparticles were neutralized, indicating that silver nanoparticle toxicity is solely mediated by free silver ions. The interactions of silver ions with the cellular components of algae differ significantly from other nanoparticle species, as it influences the particle dissolution and bioaccumulation of silver ions. However, upon exposure to silver, algae cells incite a defense mechanism which enables them to mitigate the silver toxicity and to recover over time.
concentrations in the test media, to the release of silver ions or to differences in uptake or depuration by the daphnids will be presented on the poster.

MO318
Toxicity of Ag decorated ZnO nanocrystals to Daphnia magna
C.S. Azevedo, Biology; J. Rodrigues, T. Monteiro, F.M. Costa, Universidade de Aveiro / Department of Biology and CESAM; S. Loureiro, Universidade de Aveiro / Department of Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM.

Nanoparticles (NP) are widely used in a variety of applications such as personal care products, pigments, plastics, photovoltaic cells, biosensors, etc. With the increase of their usage, research is being developed to increase or improve their applicability. An example is the combination of more than one type of NP in the same synthesis, which may change the physicochemical properties of the nanoparticles. In addition, the combination of additional nanoparticles, such as silver, to the surface of zinc oxide nanoparticles can enhance ZnO photocatalytic efficiency in a numerous applications such as in photocatalysis, antibacterial effects and gas sensors. Although the toxicity of ZnO and Ag nanoparticles has been already widely investigated, it is important to understand if as combined they will have the same effects on organisms as expected, regarding their individual toxicity. So, the aims of this work were: 1) to understand if the toxicity of Ag decorated ZnO nanocrystals was similar to the toxicity of a separated mixture of ZnO-NP and Ag-NP and 2) if their toxicity prediction can be based on the toxicity of the mixture, and consequently on the individual toxicity. To achieve that, immobilization (OECD 202), reproduction (OECD 211) and feeding inhibition tests were carried out with Daphnia magna. First, single exposures were done with ZnO-NP, Ag-NP and Ag decorated ZnO nanocrystals and then combined exposures with a mixture of ZnO-NP and Ag-NP were performed. The results of the toxicity of the Ag decorated ZnO nanocrystals were then compared with the toxicity of the mixture, and it was observed that there were some deviations from the expected, when predicting mixture and Ag decorated ZnO nanocrystals toxicity from individual nanoparticle toxicity.

MO319
Immunotoxicity and genotoxicity of pristine and purified SWCNT to the freshwater mussel Elliptio complanata
M. Revel, INRS-Institut Armand-Frappier; M. Fournier, Institut Armand Frappier/INRS / Immunotoxicology Laboratory; P. Robidoux, Universite de Sherbrooke / Aquatic and Crop Resource Development Portfolio

Production and development of carbon nanomaterials has increased for the past few years. In Canada, the forestry industry experienced an economic crisis which has led to the elaboration of new forestry products: nanomaterials, including single-walled carbon nanotubes (SWCNT). Because carbon nanomaterials have a large specific surface area, among their other extraordinary properties, they are closely studied for drug targeting and water treatment. Indeed, this property is very interesting for the removal of contaminants like metals in wastewater. Adsorption of metals is greater for purified or functionalized carbon nanotubes. Production increase of nanomaterials and applications for water treatment raises the question of their direct but also indirect toxic effects on benthic organisms which may be exposed. For example, adsorption properties of SWCNT may alter the bioavailability of contaminants present in the medium and change the global toxicity of the mixture. It is crucial to study the potential interactions between carbon nanomaterials and environmental contaminants. Immunotoxicity and genotoxicity are frequently investigated to study the effects of contaminants in aquatic environments. The immunotoxic and genotoxic measurement parameters were highly developed with bivalves because these organisms are present in a large distribution area and are in contact with various contaminants. These parameters are relevant for assessing the health of organisms and the quality of genetic information. The aim of our study is to measure the direct and indirect immunotoxic and genotoxic effect of pristine and purified SWCNT to the freshwater mussel Elliptio complanata. Our first goal is to investigate the effects of SWCNT on viability, phagocytosis and DNA strand break alone. Our second aim is to measure the same parameters in the presence of Cadmium. Results of Immunotoxicity and genotoxicity testing will be presented.

MO320
Uptake and localization of fluorescent labelled gold nanoparticles in living zebrafish (Danio rerio) using Light Sheet Microscopy
L.M. Skjolding, DTU / DTU Environment; G. Asmonaine, University of Gothenburg / Department of Biological and Environmental Sciences; R. Jolk, DTU Technical University of Denmark / Department of Microand Nanotechnology; A. Bua, DTU Environment / Department of Environmental Engineering; J. Sturve, Goteborg University / Zoophysiology Dept

Uptake and localization of fluorescent labelled gold nanoparticles in living zebrafish (Danio rerio) will be presented. Uptake of nanoparticles into cells has been shown in vitro and in vivo. However, it is challenging to find suitable methods to identify uptake and determine localization on a whole organism level. Furthermore, methods used to identify nanoparticle uptake have been associated with artefacts induced by sample preparation including staining methods for electron microscopy. This study used Fluorescent Light Sheet Microscopy (FLSM) to determine uptake and localization of fluorescent labelled nanoparticles in living whole organisms with minimal sample preparation. Two strains of D. rerio (wildtype AB and transparent Casper) were exposed to 50 nm PEG coated gold nanoparticles (Au NP) synthesized with 1% of a fluorescent probe (FITC). The fish were exposed to the particles through the diet or the water phase in a series of separate experiments. In the dietary exposure experiments Artemia salina were exposed to 1 mg Au/L for 24h before being fed to D. rerio. For exposure through the water phase 1 mg Au/L was added directly to aquaria holding the fish and non-exposed A. salina was used for feeding. Imaging of D. rerio was done after 1, 3 and 7d of uptake and again after 1 and 3d of depuration with FLSM for both dietary and aqueous exposures. The FLSM proved to be excellent for in vivo detection of NP, it was found that the fluorescence of wildtype AB zebrafish hindered deep penetration of the light thus yielding less clear images compared to the Casper strain. A time dependent increase in fluorescence was observed in the gut region of the fish after dietary exposure. No fluorescence was observed outside the gut channel indicating no or limited transfer of AuNP outside the organ. Fish exposed through the water phase showed adherence of AuNP to fins and especially gills. Strong fluorescence signal was also observed in guts of fish due to passive intake or drinking of exposed water. Fluorescence was also detectable in parts of the head which could indicate uptake into the brain through the olfactory tract. During the depuration phase the fluorescent signal decreased with time for both exposure scenarios. These results suggest that the route of uptake is pivotal for localization and transport of nanoparticles in zebrafish. Furthermore, the study shows the suitability for whole imaging of living organisms using FLSM.

MO321
Development of an in vitro tool based on the use of fish cell lines to assess the cytotoxicity of engineered nanomaterials
M. Fernandez-Cruz, INIA / National Institute for Agricultural and Food Research and Technology / Department of Environment; M. Fernandez-Cruz, INIA / National Institute for Agricultural and Food Research and Technology / Environment; J. Navas, INIA / Madrid

The production and application of engineered nanomaterials (ENMs) is rapidly increasing. Development of suitable models for screening possible toxic effects of ENMs is essential to promote screening at the early stage of development of the nanotechnology industry. Studying the toxicity of ENMs with in vitro systems will provide valuable information for risk assessment, and help to determine their interactions with cellular biomolecules. We have studied the effects of several ENMs in two fish cell lines (macrophages and hepatocytes), to elucidate their involvement in cellular viability and immunity mechanisms. The ENMs tested were the pristine forms of CuO (used as antimicrobial wood coating), WCCo (a ceramic material that improves the hardness of the products), Multi-Walled-Carbon-Nanotubes (MWCNTs; used as antifoaming bio-adhesion and coating lightweight, compact plastics), Organic Pigment Red 254 and FeO2 (coloured plastic composites). Cells exposed to CuSO4, CoCl2 and Na2WO4 salts washed several times with controls. Both cell lineages involved different types of ENMs in cell culture media was established. The effectiveness of this method was evaluated by measuring the stability and hydrodynamic size of particles by Dynamic Light Scattering, at 0h and after 24h of incubation under culture conditions. Stable dispersions were obtained, except for CuO, where some aggregation is detected. MWCNTs yield a polydisperse pattern that is maintained after 24h of ENMs exposure, evaluating simultaneously three different endpoints that cover three mechanisms of cytotoxicity: metabolic activity (using the Alamar Blue assay), plasma membrane integrity (CFDA-AM assay) and lysosomal functionality (NR assay). The involvement of ENMs in cell viability was also studied at subcellular level by electron microscopy. Cytotoxic effects (cell viability below 80% of control levels) were reproducibly produced by CuO (25 µg/ml) and MWCNTs (12.5 µg/ml), at both, metabolism and membrane levels, respectively. The rest of ENMs show negligible effects on cell viability. The implementation of these in vitro, short-term cytotoxicity assays for testing different nanomaterials is the first step for the development of predictive tools that allow estimating the possible toxicity of ENMs in more complex (in vivo) systems. Acknowledgements - This research was supported by FP7-SUN project (Grant Agreement No. 604305).

MO322
Association between aqueous phase nanoparticles (TiO2 and SiO2) and dissolved Cu2+ assessed by evaluation of Cu2+ bioavailability in zebrafish. Danio rerio, Heriot Watt University / School of Life Sciences; J. Kalman, School of Life Sciences; T.F. Fernandes, T.B. Henry, Heriot-Watt University / School of Life Sciences

Environmental concerns over engineered nanoparticles (NPs) include the potential that NPs can associate with other substances (e.g., toxicants) and these associations lead to changes in NP physicochemistry, environmental fate, and toxicity of the NP and the associated substances. Some NPs present a negative surface charge in the aqueous phase and dissolved cation (e.g., toxic metal ions) may become associated; however, there is little information on the changes in NP physicochemistry or cation bioavailability after these associations occur. The objective of this study was to investigate whether copper ions (Cu2+) associate with negatively charged NPs in the aqueous phase, impact NP physicochemistry, and lead to changes in Cu2+ bioavailability in larval zebrafish, Danio rerio. Initial work focused on TiO2 NPs, P25, and a 1 g/L stock of TiO2 NPs was prepared in deionized water and sonicated twice for 8 min at room temperature before dilution to 1-10 mg/L and evaluation of NP physicochemistry by dynamic light scattering and nanoparticle tracking analysis. The potential for Cu2+ to associate with TiO2 NPs, P25, and a...
was determined by evaluating changes in the concentration-response (mortality) curve for larval zebrafish exposed to Cu\(^{2+}\). Parallel Cu\(^{2+}\) dilutions (0–410 \(\mu\)g/L) were prepared, 1 \(\text{mL}\) of TiO\(_2\) NPs (non-toxic based on previous experiments) was added to one dilution series, and mortality of zebrafish was assessed from age 96–108 h post fertilization. Larvae exposed to sub-lethal Cu\(^{2+}\) concentrations were collected for assessment of metallothionein (MT2) gene expression. For 5 \(\text{mL}\), TiO\(_2\) NP, the \(z\)-potential was -6.45 mV and the hydrodynamic diameter was 522 ± 160 nm (PDI=0.5) (mean ± SD). When larvae were exposed to Cu\(^{2+}\), the 48-h lethal concentration (LC\(_50\)) was 185 \(\mu\)g/L, and when 1 \(\text{mL}\) of TiO\(_2\) NPs were present the acute toxicity of Cu\(^{2+}\) was reduced (LC\(_50\)) was 138 \(\mu\)g/L. Results support the hypothesis that TiO\(_2\) NPs have a negative surface charge in the aqueous phase, and that Cu\(^{2+}\) ions associate with TiO\(_2\) NPs and become less bioavailable. Ongoing research will investigate changes in TiO\(_2\) and TiO\(_x\) NP physicochemistry upon interaction with other substances and how these associations influence substance bioavailability. This research is supported by EU FP7 Future Nano Needs, “Framework to respond to regulatory needs of future nanomaterials and markets”.

**MO323 Ecotoxicology of Gold Nanomaterials: Effects on periphyton, L. stagnalis, and H. Azteca in an aquatic food chain study**

M. Hudson, University of Michigan / School of Natural Resources and Environment; A. Burton, University of Michigan / School of Natural Resources Environment; D. Costello, Kent State University / Biological Sciences

The field of nanomaterials has been rapidly expanding and, in particular, gold nanomaterials (AuNMs) are of special interest because of their growing number of applications in medicine, therapeutics, cosmetics, sensing, catalysis and the electronic industries. This expanding use of nanomaterials could pose a serious concern to aquatic environments and research into the toxicity and potential trophic transfer of nanomaterials at environmentally relevant exposure concentrations is needed. This study investigated the uptake and movement of AuNMs through an aquatic food web. Field-collected (Huron River, Ann Arbor, Michigan, USA) and lab cultured (Oscillatoria) periphyton were exposed to AuNM in a closed recirculating flume system with three treatments: control (0 \(\mu\)g/L), low (100 \(\mu\)g/L), and high (500 \(\mu\)g/L), respectively. AuNM quickly aggregated and precipitated from the water column and gold was measured in periphyton. *Hydella azteca* and *Lymnaea stagnalis* were then exposed to periphyton from the flumes for 24 h before being transferred to clean systems for a 24 h depuration period. Gold was detected in *L. stagnalis* (high average 2.3 \(\mu\)g/L and low average 1.8 \(\mu\)g/L dry weight) but not in *H. azteca*, which may have been attributed to their unique feeding mechanisms. No significant mortality or biomass modification in either *L. stagnalis* or *H. azteca* was observed. The results suggest that the trophic transfer of AuNM is organism specific and that selective feeding by macroinvertibrates, and the settling and aggregation properties of AuNM are important when considering its fate in the environment and movement through aquatic food webs.

**MO324 The cytotoxic effects in human prostate carcinoma cell line (22Rv1) exposed to various sized silver nanoparticles**

J. Kang; N. Hong, Chonbuk National University; Y. Jung, R. Kim, Korea Institute of Toxicology; J. Park, Korea Institute of Toxicology / Center for Environmental Biotechnology

Silver nanoparticles (AgNPs) are widely used in a variety of commercial fields because of their strong antibacterial activity. In many previous studies, the cytotoxic effects of AgNPs have been investigated. In this study, we exposed three different sized AgNPs (10, 20 and 50 nm) to different sized silver nanoparticles. The cell viability and ROS generation on 22Rv1 cells were determined by evaluating changes in the concentration of reduced glutathione (GSH) and the activity of superoxide dismutase (SOD). CuO NPs were more toxic towards human prostate cells compared to AgNPs. Additionally, the accumulation around brain, ovary and testis of rat was recently reported. In this study, we exposed three different sized AgNPs (10, 20 and 50 nm) to human prostate carcinoma cell line (22Rv1) and measured the cytotoxic effects such as cell viability, reactive oxygen species (ROS) generation and change of calcium ion concentration. The cell viability and ROS generation on 22Rv1 exposed to AgNPs were size- and dose-dependent. The cell viability was the lowest at 10 nm AgNP (AgNP-10) whereas the highest at AgNP-50. Also, the highest level of ROS was observed at AgNP-10 whereas the lowest was did at AgNP-50. Besides, we investigated the potency of endocrine disruption of AgNPs by measuring the luciferase activity induced by binding between androgen and androgen receptor, from which the reduced luciferase activity. These results suggests that a long-time exposure of AgNPs in human can cause the toxicological effects in the reproductive or endocrine systems.

**MO325 The non-invasive continuous monitoring of sub-toxic oxidative stress triggered by nanoparticles in the flounder, Platichthys flesus using an optosensor**

N. van Moos, University of Geneva, Institut F.A. Forel / Institut FA Forel; V. Koman, C. Santschi, EPFL, Swiss Federal Institute of Technology in Lausanne; V.I. Slaveykovka, University of Geneva / Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences

Thanks to its photoactivity and opacity, nano-TiO\(_2\) is one of the most common engineered nanomaterial found both in industrial applications as well as in numerous consumer products. Its release into natural aquatic systems is inevitable. Its toxicological hazard mainly lies in its photocatalytic activation by UV, the generation of reactive oxygen species (ROS) and subsequent oxidative stress. Though the current knowledge base suggests moderate overall toxicity of nano-TiO\(_2\) towards aquatic microorganisms, further monitoring and investigation is nonetheless required to elucidate its sub-toxic and especially chronic effects. The aim of this study is to follow the dynamics of extracellular and intracellular ROS generation within 1 h, elucidate the modifying effects of exposure conditions (exposure concentration, medium, UV) on the sub-toxic effects of nano-TiO\(_2\) as well as to compare the results obtained by two different, ROS detecting techniques via UHPLC-DAD and Cytosensor. By integrating the data obtained this way, we aim to both contribute to a better understanding of the underlying toxicity mechanisms of NPs towards aquatic microorganisms as well as to show the applicability of a promising new method for the non-invasive, real-time and continuous investigation of sub-toxic oxidative stress in AMOs. Results suggest that environmental matrices may mitigate ROS-inducing effects at the tested exposure concentrations. Furthermore, we found that nano-TiO\(_2\) exert sub-toxic effects to *C. reinhardtii* despite the heavy agglomeration observed in the exposure media at physiological pH. What is more, the biosensor seems to provide a higher sensitivity towards ROS levels than the fluorescent probes used for flow cytometry and as such may lend itself well to the mechanistic investigation of sub-toxic, cellular effects by a new approach.

**MO326 The marriage of biological systems with carbon nanomaterials: Systematic investigations of interactions and induced mechanisms**

C. Dinu, R. Eldawal, West Virginia University / Chemical Engineering; Y. Rosland, West Virginia University / Basic Pharmaceutical Sciences

Carbon nanomaterials have been explored for a wide variety of applications from fiber optics, to conductive plastics, and from composites to molecular electronics. More recently, their “bottom-up” functionalization with proteins, carbohydrates, or nucleic acids opened up exciting research directions in biolabeling, biodetection, biomolecule delivery, bioseparation or regenerative medicine and tissue engineering. This research was supported by different research directions in basic laboratory research in Dinu’s Lab at West Virginia University; aspects related to the nanomaterial-biological molecule nanointerface reactions, nanomaterial-induced toxicity and cellular biophysical changes, as well as nanomaterial-induced effects on the cell-cell interactions and nanomaterials-induced toxicity will be discussed. The goal is to provide a systematic analysis of the “good, the bad and the ugly” insights of carbon nanomaterials and means to understand and control their interface with biomolecules and cellular systems for applications ranging from molecular diagnostics to environmental protection.

**MO327 Influence of copper oxide nanoparticle shape on bioaccumulation, cellular uptake and effects in the estuarine sediment-dwelling polychaete, *Nereis diversicolor***

A. Thit Jensen, Roskilde University / ENSPAC; A. Dybowska, Natural History Museum London / Mineralogy; C. Kobler, Technical University of Denmark; G. Kennaway, Natural History Museum / Imaging and Analysis Centre; H. Selck, Roskilde University / D. Hernandez, Environment; E. Conde, M. Hernandez, SVI Switzerland for Research and Technology / Environment; D. Hernandez, Swiss Federal Institute of Technology in Lausanne; L. Li, Research Center for Agricultural and Food Research and Technology / Environment

Copper oxide nanoparticles (CuO NPs) released into the aquatic environment will likely accumulate in the sediment, where sediment-dwelling organisms may be exposed. Here we synthesized, characterized and studied bioaccumulation, internalization and effects of CuO NPs with different shapes: Spheres (CuO-S), Rods (CuO-R) and Spindles (CuO-Sp) in the benthic ragworm *Nereis diversicolor*. Copper was exposed for 10 days to two different sediment concentrations: 0.39 and 50 \(\mu\)g Cu\(^{2+}\)/d (0.39 \(\mu\)g Cu\(^{2+}\)/d). All Cu NPs were accumulated in worms in a concentration dependent manner. Only Cu-Ag resulted in avoidance behavior, suggesting that worms can detect and avoid aqueous Cu in sediment, but not CuO NPs. Transmission electron microscopy of gut sections indicated limited presence of CuO NP-like objects in the gut. Whether accumulated Cu from CuO NP exposure was internalized as particles or was still unclear. Overall, Cu form (Cu-Ag vs CuO NP) and exposure concentration may be more important for bioaccumulation and adverse effects than CuO NP shape.

**MO328 Enhanced toxicity in human and fish cell lines after co-exposure to Cu nanoparticles and non-toxic concentrations of ZnO nanoparticles.**

D. Hernandez-Moreno, INIA / Environment; L. Li, Research Center for Eco-Environmental Sciences; M. Connolly, INIA National Institute for Agricultural and Food Research and Technology / Environment; E. Conde, M. Hernandez, CIEMAT; M. Hernandez, Universitat München / Chemistry; J.M. Navas, M. Fernandez-Cruz, INIA - National Institute for Agricultural and Food Research and Technology / Environment

The main goal of this study was to evaluate the response of a human and a fish hepatoma cell lines ( HepG2 and PLHC-1, respectively) to the coexposure of increasing concentrations of a copper nanoparticle (CuNP) and a non-toxic concentration of zinc oxide nanoparticles (ZnO NPs) after a 48 h exposure. Cell lines were exposed to a 50 nm CuNP at a range of concentrations (0.39-25.0 \(\mu\)g/mL) alone or together with a non-toxic concentration (6.25 \(\mu\)g/mL) of two different ZnO NPs (25 and 100 nm). To establish the contribution of Zn ions to the toxicity caused by ZnO NPs, cells were also co-exposed to CuNPs and to supermatants
MO329 Evaluation of a new test design for the determination of a substance specific plant uptake factor (PUF) for use in regulatory fate modeling

M. Lamsheef, Bayer CropScience AG; RD: H. Ressler, Syngenta Agro GmbH; G. Reinken, Bayer CropScience AG; C. Schriever, BASF; S. Schubert, Dow Agro Sciences LLC; J. Webb, S. Webb, The institute of environmental and human health; B. Zilgern, DuPont de Nemours Deutschland GmbH; D. Keenan, Ricerca Biosciences; W. J. Doucette, Utah State University / Utah Water Research Laboratory; G. Fent, RLP Agroscience; V. Gourlay, Rheinland-Pfalz AgroScience GmbH; K. Malekani, Smithers Viscient / Environmental Fate and Metabolism; S.P. McLaughlin, Smithers Viscient / Department of Environmental Fate; P. Rooney, W. van Beinum, FERA; B. Rieder, Eurofins AgroScience; M. Traub; S. Swales, Smithers Viscient ESG; K. Weinfurner, K. Derz, IME Fraunhofer, K. Kemmerling, VCI; M. Letourneau, J. Martin, N. Marchessault, Smithers Viscient

In recent years the estimation/quantification of uptake into plants of plant protection products (PPP) has received increasing attention as the process reduces the quantity of PPP and their metabolites available for leaching. The plant uptake factor (PUF) accounts for this process in the commonly used fate models for leaching (e.g. FOCUS PEARL, FOCUS PELMO and FOCUS MACRO). However, an approved protocol for a standardized test design is not yet available from official bodies such as the OECD and consequently the level of acceptance of experimentally derived PUF values differs across various authorities. The German Crop Protection Pest Control and Fertilizer Association (IVA) as well as the European Crop Protection Association (ECPA) have made proposals in the past of how to derive the PUF from simple laboratory studies. These proposals have been discussed with stakeholders and a new study design has been developed for deriving plant uptake factors for plant protection products to be used in regulatory leaching models. The IVA/ECPA working group “PUF” has therefore coordinated a ring test to evaluate the new protocol which has taken into account improvements that were suggested by academia and regulatory authorities during a workshop held in York (UK) in September 2013. The workshop was conducted to establish a common understanding of plant uptake science and its implementation in the leaching models. Ten participating organizations conducted a ring test based on the new protocol to derive plant uptake factors for [\(\text{Cu}\)]-1,2,4-Triazole (conc. 100µg/L) in wheat by means of a hydroponic system. During eight days of incubation data on the uptake (NRU) assay. In order to obtain additional data, to clarify the mechanistic potential of the increased toxicity in the co-cultures, the presence of increasing mass of ZnONPs suspensions. Viability of cells was evaluated by three different cytotoxicity assays, the tetrazolium salt reduction (MTT) assay, the CFDA-AM assay and the neutral red uptake (NRU) assay. In order to obtain additional data, to clarify the mechanisms of toxicity, the total intracellular copper and zinc content in both cell lines after 48 h of exposure was determined by means of inductively coupled plasma-mass spectrometry (ICP-MS). The effect of the WWTP effluent was determined using a non-toxic and the highest concentration of CuNP (6.25 and 25 µg/mL), alone or in co-incubation with ZnONPs suspensions. The co-incubation of both cell lines with CuNPs and ZnONPs increased the toxic effect observed for the CuNP alone with a more pronounced effect in the human cell line. The highest toxicity was reached with the largest NP of ZnO. There was no cytotoxic effect when co-incubation with ZnONPs were performed. FOCUS Zn ions seems to modulate the cytotoxic effect of copper, showing an enhancement of viability at all the concentrations of Cu in PLHC-1 cells and at 25 µg/mL in HepG2 cells. This result indicate that the ZnONPs is the responsible of the toxic effect and not the Zn ion. In both cell lines, the intracellular zinc content increased with the increasing CuNPs concentrations although it was not statistically significant in the fish cell line. Additionally, a clearer decrease of Cu intracellular copper concentration was observed only in the human cell line. The obtained results suggest that the presence of increasing concentrations of CuNPs increased the internalization of ZnONPs, which could be the responsible of the increased toxicity in the co-exposure experiments.

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adequately aligned to other exposure areas. The described EFSA approach is limited to PECsoil, covers only a limited number of crops and allows no use of measured wash-off refinement values. An alternative approach may be to consider effects of wash-off not by the means of insufficient harmonized FOCUS models, but canopy processes, based on a model independent calculation assuming a single realistic worst case rainfall event. In this way the additional soil loading due to rainfall or run-off could be linked to the canopy reaching the soil at day of application. Within this study detailed analyses were investigated to estimate the additional soil loading caused by wash-off of agrochemicals at European level. The spatial computation assumes standard FOCUS weather data and a simplified crop canopy model as proposed in the draft EFSA soil guidance. Effects of the weather data of different agro-climatic regions within EU on the wash-off soil loading are presented and discussed. Outcome is compared to a wash-off loading based on a single realistic worst case rainfall event. The impact of additional parameters, resulting from application timing or product label, is addressed.

MO334 A simple Approach for the Estimation of Crop Interception Values for Leaf Development Stages

D. Nickisch, Environmental Fate Modeling; U. Böttcher, Christian-Albrechts-Universität zu Kiel / Institute of Crop Science and Plant Breeding; E. Ditter, Environmental Fate Modeling

Crop interception values established by the FOCUS Groundwater Scenarios Workgroup in 2000 and used for the estimation of predicted environmental concentrations in soil and groundwater for some major crops during the life cycle of a crop. This tool is based on the same rules and suitable application dates are identified for each crop. Thus, a literature review was carried out in 2010 on behalf of EFSA to collect data for spray plant interceptions. Out of a total of 55 relevant references, not more than 20 were available for a few arable crops, and data for leaf developmental stages (BBCH 10–19) was even more limited. Surprisingly, there were even negative interception values in a few cases. The EFSA literature review showed that reliable data is not publicly available in an appropriate amount. Thus, we tested a simple approach that allows an estimation of the interception values for early crop development stages. The leaf area index (LAI) or soil cover determines to a great extent the potential fraction of the substance that is intercepted by the leaf. Therefore, we determined the soil cover by taking digital photographs for several crops (wheat, maize and oilseed rape) at early growth stages. Photographs were taken directly above the crop. Afterwards, the proportion of the green parts of the photograph was determined with an algorithm to estimate the ground cover of the crops. Since a digital photograph represents a two dimensional area, we assume that the ground cover represents a conservative estimate for the interception values. In addition, the LAI values were occasionally measured by a destructive method, representing the three dimensional area, which represents the maximum interception potential of a crop. First results confirmed that the interception values estimated by a photograph are conservative in comparison to the LAI values. The interception values varied considerably within the range given by the FOCUS guideline for BBCH 10–19. In general, a considerable soil cover could be estimated at early crop growth stages. The magnitude of soil cover at emergence depends on crop growth stage, sowing distance and sowing depth of a crop. Our simple approach represents a cost effective method for a conservative estimation of interception values for leaf development stages of all FOCUS crops.

MO335 A Pesticide Application Timer (PAT) for the FOCUS groundwater models

M. Weig, WSC Scientific GmbH / Dept. E fate Modelling; B. Kind, A. Guckland, WSC Scientific GmbH

For the registration of plant protection products in the EU a groundwater risk assessment, require the input of application dates, which will then be used in the fate model to simulate pesticide transport in the vadose zone of the soil column. Thus, a digital photograph represents a two dimensional area, we assume that the ground cover represents a conservative estimate for the interception values. In addition, the LAI values were occasionally measured by a destructive method, representing the three dimensional area, which represents the maximum interception potential of a crop. First results confirmed that the interception values estimated by a photograph are conservative in comparison to the LAI values. The interception values varied considerably within the range given by the FOCUS guideline for BBCH 10–19. In general, a considerable soil cover could be estimated at early crop growth stages. The magnitude of soil cover at emergence depends on crop growth stage, sowing distance and sowing depth of a crop. Our simple approach represents a cost effective method for a conservative estimation of interception values for leaf development stages of all FOCUS crops.

MO336 Long-Term Freundlich Sorption Coefficients Derived from Terrestrial Field Accumulation Studies

R. Su, Bayer CropScience LP / Environmental Safety

A Pesticide Application Timer (PAT) for the FOCUS groundwater models (e.g. FOCUS-PEARL 4.4.4, FOCUS-PELMO 5.5.3) used in the risk assessment, require the input of application dates, which will then be used for each year of the simulations. Consequently, some applications might occur with bad weather conditions, i.e. heavy rainfall on day of application or on the following days. The modelling contradicts with good agricultural practice, as farmers would choose more appropriate dates. As the timing of rainfall on the days following application influences the amount of the pesticides washed off from plant surfaces, it is important to take into account the weather conditions during the groundwater contamination. In the surface water assessment, this problem was solved by inventing the "Pesticide Application Timer" (PAT) in FOCUS-SWASH, which selects the application dates on the basis of certain rules. The user provides a time window in which the application(s) should occur. For groundwater modelling, however, no such tool is available up to date. Therefore we developed such a tool, which is capable of identifying realistic application dates for groundwater modelling. This tool is based on the same rules and suitable application dates are identified for each year. These dates can be entered in the groundwater models ensuring that this procedure corresponds well with the good agricultural practice of farmers. We show that groundwater concentrations can be considerably different when using realistic vs. unrealistic application dates. The magnitude of these differences depends on substance properties, scenario and climate.

MO337 Modelling and experimental evidence of low pesticide persistence in the context of PBT assessment

B. Jene, BASF SE / Environmental Fate

Substance persistence needs to be evaluated for PBT assessment which is required under different regulations for chemicals, biocides and pesticides. For pesticides the REGULATION (EC) No 1107/2009 does not permit EU registration of substances that meet all three PBT criteria. If two out of three criteria are met the substance will become candidate for substitution (CIS) with limited registration time (only seven years) and comparative assessment on product level in the member states. A crucial criterion for the reference temperature is the half-life under ambient conditions. This half-life should characterize a substance as persistent in soil and sediment (DT50 > 120 days) or fresh water (DT50 > 40 days). Whereas for pesticides the DG SANCO working document on POP, PBT and vPvB requires a reference temperature of 20 °C for industrial chemicals and biocides the DT50 values are usually normalized to a reference temperature of 12 °C. Using a Q10 value of 2.58 (EFSA, 2007) extrapolation from 20 °C to 12 °C results in a 2.1-fold higher half life and substances that are considered not persistent at 20 °C could be labelled as persistent at a reference temperature of 12 °C. In the context of harmonization of the PBT criteria amongst different legislations it should be evaluated which reference temperature is appropriate to characterize substance persistence. Regarding the history of the criteria setting it can be assumed with certainty that a reference temperature of 20 °C (room temperature) is consistent with the original studies with which those substances against the threshold values were benchmarked. In a modelling exposure assessment in soil it can further be shown that for substances with soil half-lives between 56 and 120 days (not P with $T_{50}$ > 20 °C but with $T_{50}$ = 12 °C) extrapolation to relevant outdoor temperatures does not result in any
accumulation of the substances even after regular multi-year application. After terminating pesticide application these substances will be completely degraded after a few years. By analyzing multi-year experimental accumulation studies under real agricultural use conditions of concerned pesticides (DTSO₉₅, 56 – 120 days) it was found that the maximum soil concentration did not further increase after a few years of application (2-3 years). The maximum concentration found after multi-year application was not greater than the initial concentration after the first application and a pronounced decrease of the concentration between the applications was measured in all cases. It can therefore be concluded that a reference temperature of 20 °C linked with the trigger half-lives of the PBT P- assessment would not only be consistent with the history of the setting but also sufficiently discriminating to identify substances of concern. Setting the reference temperature to 12 °C would result in p-labelling of many substances that do not show any concerns regarding accumulation in the environment (false positives).

MO334
Mixture-toxicity exposure assessment in FOCUS surface water scenarios - Development of a software tool and implementation of a promising risk assessment approach into FOCUS. D. Weber, Eurofins Regulatory AG / Bio V; G. Eck, Eurofins Regulatory AG
The registration of plant protection products in the EU requires a standard risk assessment for aquatic organisms, where toxicity endpoints are compared to PEC₉₅ (Predicted Environmental Concentrations in surface waters). The FOCUS model calculations use realistic worst-case scenarios, where pesticides can enter surface water via spray drift, runoff or storm drain. Simulations result in complex exposure patterns with multiple peaks over long periods. An aquatic risk assessment for mixture toxicity is required for formulations that contain two or more active substances. A common known approach is to evaluate combined effects based on Toxicity/Exposure Ratios for all actives. This approach evaluates the cumulative risk and conservatively assumes a simultaneous occurrence of exposure peaks. However, the FOCUS surface water scenarios often show exposure patterns where occurring maximum concentrations differ in time for active substances, when applied with identical crop/scenario/application timings. A detailed analysis of the ‘realistic worst-case’ exposure profiles for each of the actives is a promising refinement option for critical scenarios. A software tool was developed that allows the user to perform a step-wise evaluation of mixture toxicity in FOCUS surface water scenarios and for formulations containing up to three active substances. The scenarios can be evaluated individually or simultaneously in a first step, resulting in detailed results and visualizations. If scenarios fail the first evaluation step, an in-depth scenario analysis can be performed in a second step, resulting in the identification of relevant key characteristics (e.g. maximum peak duration) for the scenario-compound combinations. Detailed results and professional visualizations provide a profound basis for decision making in higher risk assessment. An example for a time-dependent additive mixture toxicity assessment is presented introducing the step-wise approach and explaining the results.

MO335
PRIMET_Registration_Ethiopia 1.1 for assessment of risks in the environment and for human health in Ethiopia. M. ter Horst, Altaria, WUR; P.I. Adrianaise, Altaria Wageningen University and Research Centre / ERA team; L. Pardon, Alterra Wageningen UR; J.W. Deneer, Alterra Wageningen University and Research Centre / ERA team; J. Vlaming, Envisa Consultancy; A. Woldeamanuel, PHRD Ministry of Agriculture Ethiopia; B. Teklu, Alterra, Wageningen University and Research Centre / ERA team; L. Wipfler, Alterra Wageningen UR; J.W. Deneer, Alterra Wageningen University and Research Centre / ERA team; H. Budzinski, University of Bordeaux / UMR EPOC Equipe LPTC; P. Pardon, University of Bordeaux / UMR CNRS 5805
A software tool was developed that allows the user to perform a step-wise evaluation of mixture toxicity and for formulations containing up to three active substances. The scenarios can be evaluated individually or simultaneously in a first step, resulting in detailed results and visualizations. If scenarios fail the first evaluation step, an in-depth scenario analysis can be performed in a second step, resulting in the identification of relevant key characteristics (e.g. maximum peak duration) for the scenario-compound combinations. Detailed results and professional visualizations provide a profound basis for decision making in higher risk assessment. An example for a time-dependent additive mixture toxicity assessment is presented introducing the step-wise approach and explaining the results.

MO336
In the context of the EU approval of copper (Cu) under Regulation (EC) 1107/2009, safe use conditions of Cu containing plant protection products could be demonstrated only for greenhouse applications. Toxicity of Cu fungicides towards soil organisms is strongly dependent on physico-chemical properties, Cu background concentrations and Cu being present are not sufficiently addressed at EU approval in order to support outdoor applications. In contrast, the method applied in the context of the voluntary risk assessment procedures of Council Regulation (EEC) No 793/93 and 1488/94 takes into account these factors and was therefore deemed appropriate for calculating regional and local predicted no effect concentrations (PNEC) of Cu in soil. The method is based on an extensive set of ecotoxicological endpoints, which are normalised against soil properties such as soil organic matter, cation exchange capacity, pH and texture. Based on these normalised endpoints, site-specific PNEC values were calculated for the relevant viticulture areas of Austria taking into account the digital Austrian soil map eBod. In this presentation, results of soil Cu monitoring programmes and calculated site-specific PNEC values will be presented and the implications discussed.
EPOC-LPTC, M. Dévier, LPTC-EPOC, University of Bordeaux / Oceanic and Continental Environments and Palaeoenvironments; D. Granger, M. Chambolle, X. Litrico, LyRe

Climate change and urbanization can be key factors affecting water quality. In this context of long-term environmental changes it is important to improve knowledge about ecosystem functioning and to characterize urbanization impacts, like water treatment plant (WWTPs) effluent discharge. The aim of this study was then to characterize Bordeaux’s WWTP effluent contamination and to evaluate its input on Garonne River contamination by pesticides. This study covered the two main Bordeaux urban agglomeration (CUB) WWTPs, Louis Fargue (LF) and Clos de Hilde (CdH) between May 2012 and March 2013. Two sites of the fluvial estuary (Bordeaux and La Réole) and one site on a small river which flows in the Gironde Estuary were characterized by a lower flow but impacted by a large WWTP (Cantinolle one), the Blanquefort River (Blanquefort) were studied between March and September 2013. A wide range of 58 pesticides including herbicides, insecticides, fungicides and metabolites was studied. 55 molecules were extracted and analyzed by a multi-residues protocol using solid-phase extraction and liquid phase chromatography. Fipronil and its metabolites (fipronil sulfone and fipronil sulfone) were extracted and analyzed by a specific protocol using solid-phase microextraction or stir-bar sorptive extraction and gas chromatography. Pesticides were detected in influents and effluents of the two main Bordeaux WWTPs. Pesticides quantified at higher concentrations were diuron, imidacloprid, terbutryn, fipronil, fipronil sulfone and carbendazim. Removals were globally very low (under 50 %) and variable in both WWTPs. Pesticides appeared to be very stable, cohering two elution peaks by HPLC. Tomato leaves were cultivated in a hydroponic system with WWTPs and they are discharged into the Garonne River. Median inputs range approximately from 500 mg.d⁻¹ (fipronil) to 5000 mg.d⁻¹ (diuron) and WWTPs can have a significant contribution to Garonne River contamination by pesticides. Main pesticides in river and estuarine samples were metolachlor and its metabolite with concentrations comprised between 10 and 250 ng.L⁻¹. Typical WWTP pesticides were also quantified but at lower levels. Nevertheless some concentrations (even low) could raise environmental concern. Fipronil for instance was detected in Blanquefort site at concentrations exceeding its predicted no effect concentration (PNEC, 0.77 ng.L⁻¹). Further investigations are then needed to document its presence in the Gironde estuary but also to understand its sources in WWTPs.

MO344 Measurement and model estimates for an insecticide in closed cropping system in Turkey

M. Yalçın, Adnan Mendes University / Faculty of Agriculture; C. AHAT, Adnan Mendes University / Faculty of Agriculture Lab Environmental Toxicology; A. Reis, B. Moni; A. Gavrek, E. CAMCI Adnan Mendes University / Faculty of Agriculture Lab Environmental Toxicology; C. Turgut, ADU

Acetamiprid is a neonicotinoid insecticide used to control sucking insects on many crops including tomatoes. Farmers prefer to use it because of the short pre harvest interval to ensure pesticide residue level under MRL values. The aim of the study to use the cascade model to predict the measured increase and decrease of residues following two foliar application to tomatoes. Tomato plants were cultivated in a greenhouse environment with two different WWTPs and they are discharged into the Garonne River. Median inputs range approximately from 500 mg.d⁻¹ (fipronil) to 5000 mg.d⁻¹ (diuron) and WWTPs can have a significant contribution to Garonne River contamination by pesticides. Main pesticides in river and estuarine samples were metolachlor and it metabolites with concentrations comprised between 10 and 250 ng.L⁻¹. Typical WWTP pesticides were also quantified but at lower levels. Nevertheless some concentrations (even low) could raise environmental concern. Fipronil for instance was detected in Blanquefort site at concentrations exceeding its predicted no effect concentration (PNEC, 0.77 ng.L⁻¹). Further investigations are then needed to document its presence in the Gironde estuary but also to understand its sources in WWTPs.

MO345 European-wide ecological scenarios for the exposure assessment of chemicals in aquatic systems

A. Di Guardi, M. Morselli, University of Insubria / Department of Science and High Technology; G. Morabito, Institute for Ecosystem Study Italian National Research Council; P.J. van den Brink, Alterra and Wageningen University; F. De Laender, Université de Namur ASBL / Laboratory of Environmental Ecosystem Ecology

The increasing need of enhancing ecological risk assessment (ERA) recently led to a number of attempts to introduce more ecological relevance and realism in both exposure and effect assessment. For example, on the exposure side, this was pursued by incorporating compartments and subcompartments describing primary producers and particulate/dissolved organic carbon (POC/DOC) into fate models for aquatic systems. However, the lack of coherent measured data to parameterize the small shallow lake for which measurements were proposed with regard to their resolution and accuracy suggests the need for environmental and ecological scenarios to be adopted for ERA purposes. In this work, 5 scenarios representative of European lentic shallow water bodies were developed, including information on water temperature and fluxes, phytoplankton biomass and POC/DOC concentrations. Such scenarios were developed using a two-step approach. First, we considered the case of Lake Candi, a small shallow lake for which monthly biological observations were available for 25 years, as a typical scenario for Northern Italy. Next, we adapted the parameters characterizing this reference scenario according to a climatic gradient to produce datasets for other species. We then compared these 4 scenarios, reconstructed from the reference scenario, to data found in the literature on water bodies located in the considered range of climatic conditions. The 5 scenarios were used to run the spatially-resolved dynamic model "ChimERA fate", in order to assess the influence of environmental and ecological conditions on chemical fate and exposure. One-year simulations of emissions to a phytoplankton-dominated pond were performed for two chemicals differing in hydrophobicity and persistence. Two sets of simulations were performed: (1) considering the same water treatment plant and water regime for all scenarios and investigating the differences in bioavailable concentrations among scenarios and season and (2) adopting scenario-specific emissions and water regimes and comparing bioavailable concentrations among scenarios in an absolute fashion. Results allowed making considerations about the influence of specific environmental and ecological characteristics on exposure levels and identifying the high-risk conditions for aquatic ecosystems.

MO346 Predictive Performance of Coastal Fate Model for Multimedia Environment (CFM-ME)

S. Kim, Department of Marine Science, College of Natural Sciences, Incheon National University / Department of Marine Science; Y. Kim, Greenco Inc.; D. Chae, Department of Marine Science College of Natural Sciences Incheon National University; I. Kim, Incheon National University / Department of Biology Coastal wetland is one of the most sensitive regions to the global climate-change. We developed a Coastal Fate Model for Multimedia Environment (CFM-ME), which is a multi-box dynamic multi-compartmental model to simulate the fate of hydrophobic organic chemicals (HOCs) in coastal region including intertidal wetland. Incheon coastal region, the western mid-part of Korean peninsula, was chosen as a case study region for modeling and field measurement. The CFM-ME model covers sub-tidal zone, inter-tidal zone, and inland watershed zone in space, and simulates the semidiurnal tidal change (i.e., ebb and flood-tidal flows) by which the exposed area of tidal flat changes. To evaluate the predictive performance of CFM-ME model, the concentrations of PAHs were determined for air (gaseous-phase), seawater (dissolved and particulate phases), sub-tidal sediment, inter-tidal sediment, and soil every season during one year. Thereafter, the relative concentrations (Pred_C × versus Meas_C), which was proposed to use to avoid the problems of uncertainties or lack of emission estimates, were compared between predicted and measured ratios for model evaluation. In this study, we present (1) the differences of PAHs fate in sub-tidal v.s. inter-tidal zones, and (2) the results of sensitivity analysis and uncertainty analysis for main processes and/or parameters to influence chemical fate and transport in each zone. As a next step, this model will predict and assess the effect of climate-change on environmental pollutant fate in the coastal wetland.

MO347 Which surface water pH is relevant for Risk Assessment of pesticides in Europe?

S. Reinders, Bayer CropScience / Environmental Safety Ecotoxicology; M. Ebeling, Bayer CropScience AG EnSa-ETX-TV / Environmental Safety Ecotoxicology; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment

The behaviour of the active ingredients (AIs) of plant protection products in surface waters is often influenced by the pH of the surrounding media. However, comprehensive European-wide information about relevant pH-levels of aquatic ecosystems potentially receiving AI inputs is lacking. Given this lack of data, a realistic worst-case for regulatory approaches can currently not be established without considering an uncorrelated, rather wide range of pH values as potentially relevant. The aim of the present study was therefore to determine, which ranges of pH-values are realistic and relevant for surface waters actually receiving AI input. For our study we have searched for pH-values from surface waters all over Europe (both standing and flowing) where at least one AI has been detected at least once during either an official surface water monitoring campaign (governmental data) or as part of a study whose aims were not focused on AI-related material, the affiliation of the sampling site to a freshwater ecoregion, or the photosynthetic activity of macrophytes. The range of field relevant pH-values compiled by the present study provides a reliable basis for an adequate consideration of this parameter in modelling, or fate and effect assessment experiments in the context of the regulatory risk assessment, but also academic research, for plant protection products in Europe.

MO348 Exploring the influence of environmental and ecological dynamics on exposure predictions: a modelling approach
M. Morselli, University of Insubria / Department of Science and High Technology; M. Scacchi, University of Insubria Como / Department of Science and High Technology; M. Semplice, University of Turin / Department of Mathematics; F. De Laender, Université de Namur ASBL / Laboratory of Environmental Ecosystem Ecology; P.J. van den Brink, Alterra and Wageningen University; A. Di Guardo, University of Insubria / Department of Science and High Technology

In currently used approaches for ecological risk assessment (ERAs), exposure is generally calculated assuming steady-state conditions and environmental parameters and neglecting the potential role of ecological dynamics in affecting bioavailable concentrations. This variability may create a spatio-temporal mosaic of exposures, which should not be ignored when performing ERA. In this context, a spatially-resolved dynamic fate model ("ChimERA fate") was developed, incorporating spatial and temporal dynamics of emissions, environmental parameters (e.g., temperature, water fluxes, macrophyte/plantdominated biomass, DOC and POC concentrations). Results showed, for example, that for the most hydrophobic chemical, bioavailable concentrations varied up to a factor of 3 during a simulation year in response to dynamics in primary producers biomass, and in later months along the water flow direction where phosphorus biomass was present to significantly sequester chemical from water. The results presented here revealed that the ChimERA fate model could be a vital tool for the identification of those combinations of emission, environmental and ecological conditions potentially cautioning the highest risk for aquatic ecosystems.

Acknowledgement - The Chimera project is financed by the Long Range Initiative of CEFIC (www.celtic-ti.org) (project code: LRI-ECO19).

MO349
Quick Estimation of Italian Mitigation Measures for Step 4 Surface Water Modelling
P. Scandaliati, Environmental Fate Modelling; N. Boiselle, Rifcon GmbH / Environmental Fate Modelling; G. Wiedemann, Rifcon GmbH; E. Ditter, Environmental Fate Modelling

Currently, the FOCUS PECW approach to estimate the risk of plant protection products to enter surface waters via drift, runoff and drainage is widely used in the European registration process. The final step of the FOCUS approach is Step 4. In principle, Step 4 can be regarded as a higher exposure variability参透s temporal variability of exposure deriving from changes in environmental and ecological parameters (e.g., temperature and water fluxes, macrophyte/plantdominated biomass, DOC and POC concentrations). Results showed, for example, that for the most hydrophobic chemical, bioavailable concentrations varied up to a factor of 3 during a simulation year in response to dynamics in primary producers biomass, and in later months along the water flow direction where phosphorus biomass was present to significantly sequester chemical from water. The results presented here revealed that the ChimERA fate model could be a vital tool for the identification of those combinations of emission, environmental and ecological conditions potentially cautioning the highest risk for aquatic ecosystems.

Acknowledgement - The Chimera project is financed by the Long Range Initiative of CEFIC (www.celtic-ti.org) (project code: LRI-ECO19).

MO350
Development of representative groundwater leaching scenarios for greenhouse crops
p. sharma, Crop Protection; R. Jurask, DR. KNOELL CONSULT GmbH

The recently published EFSA guidance document on emissions of plant protection products emitted from greenhouses provides information on how to perform risk assessment according to EU regulation. Although an example scenario for leaching to groundwater is given, it is stated that the representativeness of the scenario on national and European level has not been established. As described by EFSA, a greenhouse scenario requires specification of crop and soil parameters as well as soil management information and (in-system) climatic conditions. Crop-specific information should include crop stage as a function of time, information on emergence and harvest dates, leaf area index, rooting depth, and crop water requirements. The standard FOCUS scenarios usually used for open-field assessments do not contain climate files that reflect conditions in greenhouses. For that reason the pertinent weather files were modified to reflect typical irrigation volumes in greenhouses at different times of the year and were subsequently parameterized with worst case application parameters. Typical water requirements for greenhouse crops were collected from experimental stations. Temperatures are typically higher in greenhouses compared to outdoor conditions. However, also indoor temperatures follow natural amplitudes (in greenhouses where temperature is non controlled). As a result it seems acceptable to use standard FOCUS temperature scenarios to conduct initial leaching assessments. The information on soil properties, as a first approach, generalized soil profiles defined by the FOCUS groundwater group were used. The leaching behavior of active substances and their soil metabolites was assessed by means of simulation runs with the leaching model FOCUS PEARL 4.4.4 for applications in greenhouses using the modified scenarios. Results were compared with simulations conducted using the FOCUS standard scenarios and the example scenario provided in the latest EFSA guidance document. In conclusion, model simulations indicate that predicted environmental concentrations in groundwater can vary by up to two orders of magnitude depending on the scenario used in the calculations.

MO351
Spatial distribution of percolate pesticide concentrations in Germany calculated with PELMO

In the registration procedure for plant protection products in Germany the groundwater risk assessment for active substances and their metabolites at tier 1 and 2 since 2012 is based on the 80th percentile of predicted groundwater concentrations from the FOCUS Hamburg scenario and in some cases the FOCUS Kremsmünster scenario simulated over 20 years with the FOCUS PELMO model. Results from the Europe-wide FOCUS analysis indicate, that primarily the FOCUS Hamburg scenario represents ‘realistic worst case’ soil and climate conditions from arable lands in terms of chromatographic flow in soil. A new GIS based analysis was conducted with PELMO using national geo-datasets with high spatial and temporal solution to assess the representativeness of those FOCUS groundwater scenarios with respect to German climate and soil properties. The spatial distribution of percolate pesticide concentrations in Germany in 2012 was compared with the new GISPELMO over 20 years for 3348 different soil weather combinations, for different substance properties referring to sorption and degradation in soil and for different crops and application times. Based on preliminary results it can be concluded that the FOCUS Hamburg scenario is not representing the 80th spatial percentile of pesticide percolate concentrations in 1 m soil depth for German soil and weather conditions. This might be essentially affected by less organic carbon contents in widely spread soil profiles in German arable land. Vulnerable locations identified with GISPELMO, which appeared to be nearly independent on pesticide or crop properties, could be the base for selection of alternative scenarios which may better describe the percolate concentrations for the German groundwater risk assessment than the FOCUS Hamburg scenario. Such recommendations will be discussed against the background of the FOCUS scenarios used in the 80th percentile selection. And finally, simulations with a tool like GISPELMO could more precisely guarantee to always select the same targeted spatial and temporal percentile of percolate concentrations in the groundwater risk assessment for different plant protection products, crops and application patterns.

MO352
Consideration and parametrisation of photodegradates in PECgw simulations with FOCUS-PELMO 5.5.3 in the risk assessment within the authorisation of plant protection products
J. Priegnitz, Umweltbundesamt / FG IV Pflanzenschutzmittel; R. Herr, Federal Environment Agency / Section IV Pesticides and Agroecology; M. Semplice, University of Insubria Como / Section IV Pesticides, Agroecology; G. Wiedemann, Rifcon GmbH / Environmental Fate Modelling; N. Boiselle, Rifcon GmbH / Environmental Fate Modelling; M. Scacchi, University of Insubria Como / Section IV Pesticides and Agroecology

The groundwater risk assessment of an active substance includes all transformation products formed in relevant amounts according to SANCO/2221/2000 rev.10-final. If degradation pathways were not included in the soil photolysis pathway in the ground

water risk assessment especially regarding photodegradates in a tiered approach were provided by Federal Environment Agency Germany (UBA) to other authorities in EU member states and applicants, involved in the process of authorisation of plant protection products, with a call for annotation. These
recommendations have the intention to support the EU harmonization process in the risk assessment of plant protection products to ensure an agreed approach in future. With FOCUS-PELMO 5.5.3 it is possible to model both the photodegradation of an active substance and the biodegradation of the active substance and its photodegradates/metabolites in one run. There is no need to simulate a fixed apportionment of the total application rate within two virtual pathways. The model keeps track of the increasing depth of the modelled molecules and considers the photolysis only in the upper 1 mm of soil. Soil photolysis is driven by its own parameters like photolytic rate constant and the corresponding reference irradiance. EFSA guidance (2014) describes how to derive separate rate constants for surface processes and biodegradation in the soil matrix from field studies. From field dissipation studies evaluated with biphasic kinetics, the degradation rate constant of the fast phase can be assessed as representative for the soil photolysis of non-volatile substances. The reference irradiance at the application time and location of the field trial can be retrieved online from NASA. NASA provides extensive, global satellite data, daily averaged from July 1983 up to recent time. Prospective field studies should provide trial specific measured irradiance data.

MO353
An updated landuse dataset for use in European regulatory environmental risk assessments
G. Hughes, H. Lyons, Cambridge Environmental Assessments
Spatial analyses as well as spatially distributed environmental fate simulation modelling are increasingly used to refine estimates of pesticide exposure within existing models. An example of the UK Higher Tier Drainflow approach as well as the NL GeoPEARL approach. The framework encompasses 15 soil classes and 4 climate zones. The soils classes reflect: 7 soils classes that are free draining and would contribute to groundwater; 4 soils classes that contribute to groundwater at 1m depth, but would ordinarily have agricultural drains; 2 soils classes that are peaty and the leaching of PPPs to groundwater would not be expected; 2 soils classes overlying substrates that are largely impermeable and the leaching of PPPs to groundwater would not be expected; 4 weather stations were selected to represent the climate zones of 500 mm, 600 mm-750 mm, 750 mm-1000 mm and 1000 mm rainfall. The seven free draining soils classes were modelled using PEARL while the four drained soils were modelled using MACRO. Peaty soils were assumed not to contribute to groundwater contamination along with soils overlying an impermeable substrate. These climate/soil scenario combinations, when combined with the area that they represent were used to define a cumulative frequency distribution akin to that produced by FOCUS. Example outputs from FOCUS focus on the local scale and a regional scale. For wide dispersive uses the local scale focusses on the municipal STP of a standard town containing 10,000 inhabitants with releases to water assumed to go via the municipal STP and subsequent release into an adjacent river. Direct release to air and soil from wide dispersive uses is not assessed at the local scale, but at the regional scale. The regional scale is assumed to be a densely populated area of 20 million inhabitants. Releases (both point and wide dispersive) are assumed to be constant and continuous. While this framework may represent the environmental exposure route of many wide dispersive uses, it assumes initial release of the substance will be directly to a municipal STP, and at the local scale releases to soil or air are not relevant. For some wide dispersive uses, such as co-formulants used in plant protection products, this is not representative. Instead, exposure of co-formulants is likely to be edge of field, with potentially direct release to an edge of field water body via spray drift, drainage and runoff and, depending on vapour pressure or formulation type, direct release to soil. To address this, ECPA have developed the Local Environment Tool (LET). This tool allows an edge of field assessment to be conducted, using the FOCUS Step 2 approach (FOCUS 2003) for surface water and the R.16 approach (ECHA, 2012) for soil and secondary poisoning. The LET also combines local concentrations with regional PECs (steady state concentrations) estimated from all of the uses in the substance including co-formulant use, where regional release of co-formulants is estimated according to ECPA SPERCs, to determine a safe application rate. An overview of the approach will be presented alongside some preliminary investigations into the approach. The project ‘4FUN’ is currently allocating 4 FUN a new modelling tool for the integrated assessment of human and environmental exposures in different spatial and temporal dimensions. We demonstrate a new modelling tool for the integrated assessment of exposure to chemicals, called MERLIN- Expo, which is currently being refined and validated within the FP7 projects ‘MERLIN and Expo’. This tool provides a library of exposure models which can be flexibly combined to explore complex scenarios, coupling on the same platform multimedia and physiologically-based pharmacokinetic (PBPK) models for estimating human intake and internal exposure. The models can be used to dynamically simulate fate and exposure for all organic (e.g., PAHs, PCBs, pesticides) and inorganic contaminants. There is no limitation for temporal scale, and pollutants of concern can be selected according to various criteria. Moreover, MERLIN- Exp incorportates advanced functionalities for uncertainty and sensitivity analysis. MERLIN- Expo is demonstrated on a case study in the Lagoon of Venice, aimed at estimating environmental and human exposure to different congeners of PCBs. Specifically, the modelling framework combines bioaccumulation modelling in aquatic species, human intake modelling through the diet, together with PBPK modelling of internal exposure. Past PCB concentration trends reconstructed from dated sediment cores are used as inputs to model the time-dependent bioaccumulation in selected aquatic organisms. Then lifetime dietary intakes from contaminated fish and seafood, as well as other food items collected from literature data, are used to estimate PCB internal exposure for local population.
population. Modelling results are compared to available monitoring data on chemical concentrations in edible aquatic species and concentrations in serum of adult men in Venice area to assess the reliability and applicability of the proposed tool to real complex scenarios. Different outcomes of MERLIN-Expo can be used for regulatory purposes. For example, chemical concentrations in environmental media and biota can be compared with Environmental Quality Standards, human daily intake can be compared with given regulatory threshold for human health (e.g. Reference Dose) and environmental fate can be evaluated to avoid biomonitoring equivalents or other thresholds defined by risk assessors. This makes the tool interesting and promising for potential applications in different regulatory domains.

MO358 Differential effects of high fat diet and DDE treatment alone or in combination on metallothionein gene expression in rat tissues

R. Scudiero, Department of Biology; V. Migliaccio, R. Sica, R. Putti, L. Lionetti, University Federico II of Naples

In recent years the obesity, the result of increased calorie intake and decreased energy expenditure, has tripled in Europe. Inflammation is the major factor of obesity-related metabolic disease, associated to the generation of superoxide and endoplasmic reticulum stress. Environmental factors play a role in the onset of obesity. Persistent organic pollutants (POPs) are a wide group of pollutants that accumulate in the adipose tissue. POPs include the p,p'-dichlorodiphenylchlorodiphenylene (DDE), DDT major metabolite with the highest persistence. Metallothionein (MT) is a cystein-rich protein, induced by various physiological mediators. MT modulates the binding of essential or toxic heavy metals and the removal of hydroxyl radicals. MT expression is enhanced by inflammatory stimuli and is induced in adipose tissue during the development of obesity. In this study the effect of DDE exposure associated with high fat diet and subsequent caloric restriction on the MT expression was evaluated. Four groups of adult male Wistar rats were so treated: 1) standard diet (10% fat J/J) (N rats); 2) standard diet plus DDE (10 mg/kg b.w. by gavage) (N+DDE rats); 3) high fat diet (45% fat J/J) (D rats); 4) high fat diet plus DDE (D+DDE rats). After 4 weeks, 8 rats for each group were restricted for daily energy intake for 2 weeks. qPCR analyses demonstrated a tissue-specific modulation of MT expression: the changes in MT expression following high fat diet and DDE treatment depended on tissues, as well as the response to the caloric restriction. In tests no significant differences were observed between the 2 diets; DDE treatment led to a decrease in MT transcripts in N rats, but a strong inactivation of MT expression was only observed following the caloric restriction, in all groups of rats. In liver the major amount of MT transcripts was found in N rats; both high fat diet and DDE treatment led to a decrease in MT expression, whereas in MT treated rats the effects of diet and DDE were not additive, following the caloric restriction. In DDE-treated rats the caloric restriction had no effect on MT expression. In kidney high fat diet was not able to change MT expression, while the DDE treatment greatly enhanced MT gene expression, in both N and D rats. Interestingly, the caloric restriction had opposite effects on the 2 experimental groups: in N rats induced MT expression, whereas in rats fed high fat diet inhibited MT expression, both in the presence/absence of DDE.

MO359 Method to assess human health risks from dermal exposure to pesticides

K. Baniversová, A. Nečásová, Research Centre for Toxic Compounds in the Environment Faculty of Science Masaryk University, J. Kohoutek, Masaryk University / Research Centre for toxic compounds in the environment RECETOX; P. Cupr, Masaryk University, Faculty of Science, RECETOX / RECETOX Research centre for toxic compounds in the environment Plant protection products (PPPs) containing a wide spectrum of active substances are used to protect crops against noxious organisms. As a result, these compounds are released to the environment and they are being detected also beyond the area of application. They not only have adverse effects on target organisms, which is desirable, but also on non-target organisms, including humans. A complex process of assessing the properties and effects of active substances and PPPs precedes their releasing. However, not all the assays are strictly demanded. Further, even if the assays are carried out, their results and the methodology behind them are considered to be not comparable, when assessing exposure to these pesticides (including the dermal exposure) and human health risks resulting from such exposure, the risk assessors do not have relevant data at disposal. In this study, the dermal absorption of chlorpyrifos, as a model compound we have chosen based on the large prioritization process, was studied. An automatic system of Franz diffusion cells and the human skin ex vivo were used. The experimental design was set to obtain the information on the absorption kinetics of this compound. The absorption kinetics were used to calculate the risks resulting from the human exposure to fresh water contaminated by chlorpyrifos. Equations by US EPA for calculating the dermally absorbed dose were used. By this, a simple tool to calculate the hazard quotient to quantify the risks resulting from dermal exposure to chlorpyrifos in fresh waters, is going to be presented. The available experimental data on dermal absorption of active substances are crucial for the human risk assessment of pesticides found in fresh waters all over the world, and this will be shown on an example of our data from dermal exposure experiments. The obtained data will be discussed in broader context of current issues regarding the PPPs' dermal exposure assessment and regulatory context.

MO360 Introduction on the Agricultural Worker(Pesticide applicator) Risk Assessment and Future Plans in Korea

K. Paik, NAAS, RDA / Chemical Safety Division; J. Oh, M. Paik, NAAS RDA; A. You, National Academy of Agricultural Science; N. Cho, NAAS RDA; M. Jeong, National Academy of Agricultural Science / department of crop life safety International Division, OECD Working group on pesticide(OECD WG), especially, Risk Reduction Steering Group(RRSG), efforts to reduce the risk of the agricultural worker from pesticide exposure. Korean farmer spray the pesticide during 19.3 days per year and 3.76 hours per day. For this reason, need special efforts to protect of pesticide-spraying-farmer’s health. From 2009, Korean authority, division of Control of quality and safety of agro-materials/resources, start to assess the risk of Agricultural Worker(pesticide applicator) before registration, and announcement of the notice about establishment of Acceptable Operator Exposure Level(AOEL), Setting the pesticide exposure calculating method and risk assessment principle. From this notice, the TER(Toxicity Exposure Ratio) of some pesticides against the pesticides operator were calculated as devide AOEL by pesticide exposure dose. This tool, which were established by Korean authority, the AOEL, comparing the AOEL from pesticide exposure model(Korean Predicted Operator Exposure Model, K-POEM). When the TER default over the 1, the risk is low. But below the 1, that pesticide have risk, eventually, can not register. At present, K-POEM was modified from UK-POE, have many problem. Moreover, we assess the only pesticides operator’s risk. We start study for pesticide operators health with college, CRO and government institute. In future, try to improve and revise pesticide exposure calculating model suitable to Korean farming style. Also add to assess the risk of various worker, such as re-entry worker, green house worker, bystander, etc.

Advancements in life cycle impact assessment and footprint method development (P)

MO361 Life Cycle Assessment of the photocatalytic degradation of contaminated water

P. Cerazino, University fo Modena and Reggio Emilia; F. Bondioli, University of Ferrara / Department of Industrial Engineering; P. Neri, M. Pini, University di Modena e Reggio Emilia / Dipartimento di Scienze e Metodi dellingenieria; A. Ferrari, University of Modena and Reggio Emilia / Department of Sciences and Methods for Engineering

Waters are under increasing pressure from the continuous growth in demand for sufficient quantities of good quality water for all purposes. This highlights the need for a policy of preservation and restoration, aiming at both maintaining the existing good quality surface waters, preventing them from degradation on a long term, and improving the conditions of the whole aquatic environment by reducing industrial and urban discharges, emissions and losses of hazardous substances. In the last decades, several nanotechnology-based water treatment technologies have been developed and used to improve desalination, safe reuse of wastewater, disinfection and decontamination of water. Compared to traditional technologies, advanced oxidation processes (AOP), based on the presence of titanium dioxide (TiO2), provide an efficient alternative for water remediation. However, these processes are mainly exploited through photoelectroconverters with floating catalyst that may release “free” nanomaterials in the environment posing a comparable or worse harm to the environment compared to that solved through the photodegradation process. The aim of this study was to assess the environmental and health effects of a novel developed photoreactor where the titanium dioxide catalyst is immobilized on suitable substrates and activated with proper irradiation. The Life Cycle Assessment (LCA) was applied in order to drive the research towards the best technology and contribute to the development of a new awareness and knowledge on using titania based nanoproducts and nanotechnologies in water purification. In this study a methodological framework developed in collaboration with Emu (Swiss Federal Laboratories for Materials Science and Technology) to identify human health characterization factors based on the USETox model was applied.

MO362 Proposal of adaptation of the effect factor of the USETox methodology for environmental labelling of rinse-off cosmetic products

P. Martz, LOréal Research & Innovation / LOREAL; J. Lharidon, LOREAL / LOréal Life Sciences Direction; J. CHENEBLE, LORÉAL; j. campion, LOréal Research & Innovation

The USEtox model, developed for Life Cycle Assessment (LCA) within the framework of the joint project SETAC-UNEPICA Initiatives, is recommended (France) or is expected to be recommended (USA and EU) for calculating the environmental impact on aquatic ecosystems of certain consumer cosmetic products, especially shampoos and shower gels. In a previous work, presented at the SETAC Europe meeting in 2014, we showed that the USEtox model gives results on shampoo’s ranking that are poorly correlated with the ones given by the CDV (critical dilution volume) model, already applied for the awarding of the European Ecolabel to cosmetic products. This can be explained either by differences on the fate factor modelling and/or on the effect factor modelling. In this study, we have chosen to focus on the effect factor to better understand parameters driving these
Robust decision making on ecotoxicity of products: is USEtox ready for use or not?

G. Van Hoof, Procter & Gamble Services / Environmental Stewardship Organizatio; D. Schownake, Procter & Gamble Services Company NV; J. Dewaele, Procter&Gamble / Brussels Innovation Center; M. Stalmans, Procter & Gamble Services Nowadays it is widely accepted that decision making in sustainable product design should be based on a combination of economic, environmental and social indicators. Methods and databases underlying indicator results are still rapidly evolving. It is nevertheless paramount that decisions are based on robust information that is sufficiently stable over time. Decisions should be based on the latest science and data, as much as possible avoiding subjective decisions, taking uncertainty in the calculated scores into account. At the same time, the methods and data used should be easily accessible and understandable, so that all stakeholders can directly communicate to decision makers. When evaluating the environmental footprint of products, normalization of indicators is a useful approach to benchmark vs. a reference situation. While normalization is not free of data gaps and uncertainty, a multi-indicator approach (vs. a single score) is maintained, which helps decision-makers set priorities. This poster illustrates these challenges on the freshwater toxicity indicator (USEtox) applied on a hand sample of cosmetic ingredients. USEtox is a consensus method and a practical tool for calculation of characterization factors (CFs) for new chemicals, the method remains highly sensitive to input data interpretation and selection. Data sensitivity is demonstrated with effect data, in line with observations by Henderson et al. (2011). The lack of uncertainty information on calculated USEtox CFs appears critical when formulations are compared which are composed of data rich and data poor results. Results show that depending on data selected for the calculation of CFs, the product score ranges over 2 orders of magnitude. These constraints currently affect the application of USEtox for in market use, particularly when the most important decision (purchase intent) is based on such information.

Applicability of USEtox model for assessing ecotoxicity impact of copper as part of the product environmental footprint

F. Verdonck, T. De Wilde, ARCHE; L. Perez Simbor, K. Delbeke, European Copper Institute; P. Van Sprang, ARCHE To overcome the fragmentation of the internal market as regards different available materials, providing and communicating performance, the European Commission recommends Product Environmental Footprint (PEF) methods based on existing, widely recognised methods. The USEtox model (Rosenbaum et al., 2008) is proposed to assess the ecotoxicity for aquatic fresh water. Ecotoxicity addresses the toxic impacts on an ecosystem, which damage individual species and change the structure and function of the ecosystem. In this poster, it is assessed whether USEtox is applicable for ecotoxicity assessment of copper. Copper plays a crucial role in “closing” the loop and enabling Europe to move towards a circular economy. USEtox has several similarities with ecotoxicity and exposure assessment principles from risk assessment. However, despite metal specific LCA development published in literature and high quality data sets generated under the REACH Regulation umbrella, the ecotoxicity data set underlying the effect factor, essentiality, natural background and bio-availability were found to be insufficiently covered in the current available USEtox version 1.01. As a consequence, environmental impact of copper is therefore insufficiently reliable for a comparative assessment of products.

Managing the integrated direct and indirect life-cycle impacts of sewers and WWTPs in Mediterranean and Atlantic cities

A. Petit-Boix, Institute of Environmental Science and Technology; E. Iijo, Universitat Autònoma de Barcelona / Institute of Environmental Science and Technology - Department of Chemical Engineering; Y. Lorenzo, University of Santiago de Compostela / Chemical Engineering; M. Amores, S. McEnnis, CETAqua, Water Technology Centre; G. Villalba, Universitat Autònoma de Barcelona / Chemical Engineering Department Institut de Ciència i Tecnologia Ambientals; e. sevigne, Department of Chemical Engineering; J. Riradevall, Institute of Environmental Science and Technology ICTA Universitat Autònoma de Barcelona / sostenipra Managing sanitation infrastructures is of paramount importance in order to meet the demand of an increasing urban population. From a life-cycle perspective, the operation stage of the wastewater collection and treatment results in 2 different types of contributions to the Global Warming Potential (GWP). On the one hand, the indirect impacts consist of the energy, chemicals and materials required to pump and treat wastewater in sewers and Wastewater Treatment Plants (WWTP), respectively. On the other hand, the direct impacts of the system result from the gas emissions deriving from the wastewater degradation and sludge treatment. Among these, the most sensitive is nitrous oxide (N\textsubscript{2}O), which is produced by the denitrification of the nitrogen (N) found in the WWTP sludge. Emissions of N\textsubscript{2}O are 298 and 298 times greater than that of CO\textsubscript{2}. Despite the importance of N\textsubscript{2}O in terms of GWP, very few studies have paid attention to its formation in sewers and none of them has assessed the three gases simultaneously. In addition, of the entire urban water cycle, most attention has been placed on gaseous emissions from wastewater treatment plants (WWTP). In the framework of the LIFE Aquaevec project (LIFE/10/ENV/ES/S/520), Calafell (Catalonia, Mediterranean climate) and Betanzos (Galicia, Atlantic climate). Sampling campaigns were conducted during summer and winter in all treatment stages of the WWTPs and 4 representative sites of the sewers. This analysis will help to determine and compare the relative contribution of the GHG emissions produced in sewers with respect to those produced in WWTPs. From a life-cycle perspective, these direct emissions will be integrated to the operation phase of both systems and compared with the energetic requirements of sewers and WWTPs. Hence, these results can assist urban planners in the design of future sanitation systems and reduce the carbon footprint of the system.
accomplished during summer and winter in four selected points along the sewer network of each city. The purpose of this investigation was to study N₂O, CH₄, and H₂S formation, to identify the possible hot-spots of the network quantifying the concentrations and to establish measurement methodologies. The results show that high temperature favours gas production, as the highest concentrations were measured during summer and in Calafell: 316.7 µg L⁻¹ of methane, 18.3 µg L⁻¹ of nitrous oxide, and 3.4 µg L⁻¹ of H₂S. In addition, the wet wells were found to be the highest concentration point, as they have more turbulence than other sampling points, and can become anaerobic environments between breathing cycles. This research will highlight the contribution of these gases to the entire water cycle and it will help to apply Life Cycle Assessment for environmental analysis of the wastewater networks in order to improve the wastewater management and contribute to more sustainable cities.

MO368
SeawageLCI 1.0 - A first generation inventory model for quantification of chemical emissions via sewage systems. Application on chemicals of concern A. Gallice, Research Innovation; M. Birkved, Technical University of Denmark; S. Kech, Veolia

Lack of inventory data on chemical emissions often forces life cycle assessors to rely on crude emissions estimates (e.g. 100 % of the applied chemical mass is assumed emitted) or in the worst case to omit chemical emissions due to lack of emission data. The inventory model SeawageLCI 1.0, provides a mean for assessors to obtain fractions of chemicals emitted to the environment via waste water collection and treatment systems. The model is based on existing models capable of estimating chemical degradation in waste water collection and treatment systems and also on national European statistics concerning waste water treatment systems. By combining readily available statistics and models stemming from environmental chemistry and waste water treatment science, the SeawageLCI 1.0 model was built to simulate national specific average waste water collection and treatment systems and hence to the specific average emission fractions for organic chemicals. In order to illustrate the applicability, versatility and the general use of SeawageLCI 1.0, a case study set of 6 organic chemicals was assessed in 15 national waste water different treatment grids. The results obtained applying SeawageLCI 1.0 model reveal that it’s possible to account for many of the variation in emission quantities of chemicals, caused by variations in the chemical fate properties and in the composition of national waste water treatment grids. The results indicate that the total emission fraction of a chemical may vary as much as up to one order of magnitude across the 15 countries included in the case study. Further, the case study reveals that for most of the chemicals considered, the dominant emission route after transport in waste water collection and potentially waste water treatment is emission to surface water recipients, other environmental compartments such as agricultural soil may receive considerable loads of chemicals emitted by the national specific waste water grids. The SewageLCI 1.0 presentation and case study reveal how broad inclusion of chemicals emitted to the environment via waste water treatment grids ideally should be captured by LCA. Despite SewageLCI 1.0 simulates national and heterogeneous treatment systems, national grid models aren’t validated, it is concluded that the model provides the best possible general mean for including emissions of chemicals with wide and dispersive use in LCAs.

MO369
Accounting for land use change emissions and their timing for purpose-grown biomass in the energy sector: Review of best practices and application to a generic case study A. Gallice, Research Innovation; A. Levasseur, CIRAIG - École Polytechnique de Montréal

Life cycle assessment and carbon footprint studies traditionally do not account for biogenic carbon, because it is considered neutral for global warming in a long-term perspective. However, experts have recently shown that biogenic carbon neutrality may lead to accounting errors and biased conclusions, raising the need to develop accounting methodologies that consider biogenic carbon emissions. We have identified two issues related to the consideration of biogenic carbon for purpose-grown short-rotation biomass in the energy sector: (1) quantification of land use changes and potentially waste water treatment systems and (2) treatment of temporal aspects. Within this study, we have investigated and compared methodological recommendations for quantifying and considering the temporal aspects of LUC emissions from several international standards, guidelines, governmental policies, and research papers. From this critical review, we have identified best practices and applied them to a generic case study addressing very short-rotation poplar and miscanthus crops grown in different environments. The effects of land have been converted after the increase in biomass production to identify whether direct (dLUC) or indirect (iLUC) emissions must be considered. Different scenarios have been developed using local data and statistics. Then, the quantity of carbon emitted during land conversion for each of these scenarios have been estimated using IPCC guidelines for dLUC, and a study performed by the European Commission for iLUC. The second step was to account for the warming effect of LUC emissions during their payback time. Usually, biogenic carbon emissions are amortized uniformly over the average number of years of the rotation period or over a fixed time horizon. However, as shown by some researchers, this leads to an underestimation of the global warming impact of biogenic carbon emissions associated with LUC. We have used a Time Correction Factor (TCF) to take into account the warming impact of LUC emissions over time. For very-short-rotation poplar and miscanthus, the most likely scenario (use of abandoned agricultural lands) does not lead to LUC emissions, while the less likely scenario (use of forested lands) leads to LUC emissions of 180 gCO₂-eq/MJ for poplar, and 18 to 49 gCO₂-eq/MJ for miscanthus if amortized according to IPCC guidelines. If a TCF is used, these emissions become 178 gCO₂-eq/MJ for poplar, and 32 to 87 gCO₂-eq/MJ for miscanthus.

MO370
Spatial analysis of fertilizers application on agricultural land: a case study for Luxembourg S. Kech, Veolia; A. Gallice, Aix Marseille Université; A. Marvuglia, Luxembourg Institute of Science and Technology (LIST) / Resource Centre for Environmental Technologies CRÉTEResource Centre for Environmental Technologies CRÉTE; E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies CRÉTE

Agricultural soil is a major source of nitrous oxide (N₂O), nitric oxide (NO) and ammonia (NH₃). NO and ammonia play a key role in atmospheric chemistry, but little information is available on emissions of these gases from soils amended with organic fertilizers at different soil water contents. In this study fertilizers emissions to soil, surface and groundwater and air have been calculated for the country of Luxembourg based on data collected from national statistics and elaborated using the PestLCI 2.0 tool [1]. The spatial distribution of the crops is known for the entire country on a GIS support, therefore it was possible to spatialize also the related fertilizers emissions and characterize the territory of each commune from the point of view of environmental contamination. An Ascendant Hierarchical Clustering (HAC) algorithm has been used to classify the communes on the basis of their contamination level [2]. The determination of the centre of gravity of each cluster allowed the definition of 5 profiles of environmental contamination conditioned to the spatial distribution of the polluting substances, thus identifying spatial patterns of contamination between the communes. The most contaminated communes resulted to be Wincrange, Rambourg and Junglinster. The KH2 null hypothesis of independence between the spatial distribution of the contamination profiles and the spatial distribution of the profiles of human presence has been rejected with a p-value of 0.0084. This value has been confirmed by a p.value.mc=0.015 computed with the Monte-Carlo method with 2000 runs [3]. Therefore it is not possible to establish a correlation between fertilizers emissions and human presence. In future research, data on pesticides emissions will also be used to carry out a similar analysis, also exploring the correlation with other spatially distributed variables, such as access to healthcare facilities, economic conditions of the inhabitants, incidence of diseases, etc. Acknowledgement– The authors wish to thank Dr. Ivan Vázquez Rowe (PhD student, Universitat de Lleida) for his helpful help. [1] Dijkman TJ, Birkved M, Hauschild MZ. 2012. PestLCI 2.0: a second generation model for estimating emissions of pesticides from arable land in LCA. Int J Life Cycle Assess 17:973–986. [2] Everitt, B. (1974). Clustering Algorithms. London: Heinemann Educ. Books. [3] Hope A. 1968. A simplified Monte Carlo significance test procedure. J. Roy. Statist. Soc.(30):582–598.

MO371
Investigating the characterization and weighting schemes for footprinting K. Fang, Leiden University / Institute of Environmental Sciences CML; B. Heijungs, Leiden University / Faculty of Economics and Business Administration Department of Econometrics and Operations Research

This investigation is intended to show the hidden characterization and weighting elements in footprint analysis, but also to discuss the implications for classification and integration of multiple footprints. Our results demonstrate that each of the carbon, water, land and material footprints has two fundamentally different versions, addressing elementary flows on the impact assessment or inventory level. The impact-oriented footprints based on characterization models allow a variety of elementary flows to be characterized in a way that is scientifically robust and environmentally meaningful, while the pressure-oriented footprints based on subjective weighting factors contribute to inventory analysis with the aim of maintaining the physical meaning of elementary flows which in aggregate correspond to the pressure exerted by human activities. These two footprint categories have their own strengths and weaknesses, and complement each other. [1] Dijkman TJ, Birkved M, Hauschild MZ. 2012. PestLCI 2.0: a second generation model for estimating emissions of pesticides from arable land in LCA. Int J Life Cycle Assess 17:973–986. [2] Everitt, B. (1974). Clustering Algorithms. London: Heinemann Educ. Books. [3] Hope A. 1968. A simplified Monte Carlo significance test procedure. J. Roy. Statist. Soc.(30):582–598.
MO372
Local emissions, local impacts: how LCA may evolve to include local detailed damage estimates
M. Canini, National Research Council of Italy (CNR) / Istituto di Scienze dell’Atmosfera e del Clima; A. Ferrari, University of Modena and Reggio Emilia / Department of Sciences and Methods for Engineering; P. Neri, M. Pini, Università di Modena and Reggio Emilia / Dipartimento di Scienze e Metodi dell’Ingegneria; C. Mangia, Consiglio Nazionale delle Ricerche / Istituto di Scienze dell’Atmosfera e del Clima
LCA studies have the strength to investigate the life cycle to detect any relevant process and related emission that may impact on ecosystems and human health; but, almost all LCA has taken the shortcut of first summing the emissions over all stages and then multiplying the result by site independent impact indices (reaching at best the national or continental resolution); spatial and temporal details at the local scale impacted by site specific emissions (e.g. stack emissions) are not accounted for. Some procedures have been proposed to couple the LCA approach with methods that could provide more spatially detailed damage estimations. One is the IPA (impact pathway analysis) developed under the Externe project and following works; it aimed to track the fate of a pollutant from where it is emitted to the affected receptors (population, crops, forests, buildings, etc.). This work involves a multidisciplinary system analysis, with inputs from engineers, dispersion models, epidemiologists, etc.

MO373
Spatial differentiation? Yes, but, not only for impact characterization!
S. Le Feon, Istree Rennes; M. Pradel, Istree Clermont Ferrand / Ecotechnologies; L. Aissani,
Life Cycle Assessment (LCA) faces with spatial differentiation issues that have been discussed for years in LCA community. A 77 papers review reveals that the most commonly studied one is the lack of spatial consideration in the environmental impact assessment (orography, meteorology, land use and georeferenced population) provides a detailed space-time exposure field to estimate actual damage response. How to integrate and make a robust new procedure with the “good” of both the approach is the perspective of this work.

MO374
Life Cycle Assessment (LCA) regionalisation refers to the integration of the spatial variability that really exists to improve the result representativeness and reduce spatial uncertainties. Life Cycle Inventory (LCI) regionalisation deals with increasing the geographic representativeness modeled in the inventory. Life Cycle Impact Assessment (LCIA) regionalisation consists in taking into account spatial variability of receiving environment sensitivity to characterise some environmental consequences. Using regionalisation factors and the results of Monte Carlo simulation and regression analysis. This method is based on spatial uncertainty analysis and allows the selection of impacts, processes and regionalisation aspects that LCA practitioners need to focus on. Its relevance and applicability is illustrated by a LCA case study of biofuel pathways. The proposed methodology is stepwise. First most relevant impact categories and processes to be further investigated for regionalisation are selected according to their impact contribution and the uncertainty level by the Monte Carlo simulation and regression analysis. Next, CF uncertainty is compared to inventory one to determine which aspects between LCI regionalisation or spatialisation should be prioritised. Finally, the resulting uncertainty level is compared with the confident level required by the decision maker by applying statistical test. The regionalisation effort is estimated depending on the confident level is wait for the case study. And spatial resolutions. This methodology aims to account both the impact contribution and uncertainty to select process to be regionalised. The proposed methodology highlights the need to focus on the goal and scope at the early stages of a study to clearly identify the intended audience and their requirements regarding study quality. A dialogue with the decision maker during the study will be beneficial. This approach could be relevant for other types of uncertainty but should be adapted.

MO375
Spatio-temporal framework for life cycle analysis of bioenergy
A. Ajayebi, University of Exeter; M. Maier, The University of Exeter; M.D. Mutel, Agroscope Changins–Wädenswil Research Station ACW; X. Yan, The University of Exeter
Evaluating the environmental repercussions of bioenergy is affected by many uncertainties including those related to spatial and temporal variables. In this study we investigate the application of a dynamic life cycle analysis (LCA) methodology which takes into account both temporal and spatial variables. While numerous studies have attempted to estimate sustainability indicators, the most common approach is the perspective of this work.
A general framework for regionalized life cycle assessment calculations
C. Mutel, ETH Zurich / Laboratory for Energy Systems Analysis; K. Volkart, Paul Scherrer Institut / Laboratory for Energy Systems Analysis; S. Hellweg, ETH Zurich / Institute of Environmental Engineering

Comprehensive and global regionalized impact assessment (IA) methods, such as LC IMPACT and Impact World+, are now widely available. At the same time, many life cycle inventory databases include detailed geometrical definitions for the location of economic activities. The key remaining problem in performing regionalized life cycle assessment is matching the different spatial scales of the method and database. IA methods normally have a spatial scale derived from physical patterns in space, such as climate or topology. Inventory databases, on the other hand, are usually defined by political boundaries. We have developed a matrix formulation for four different matching scenarios. We have implemented these approaches in the open-source software Brightway2-regional [1]. The first scenario is the simplest - no matching is needed if the regionalized IA method and inventory database share the same spatial scales. In this case, the modification to standard LCA matrix math is relatively minor, and no GIS calculations are needed. The second scenario is to allocate inventory spatial units to IA spatial units based on intersected areas. Area is a relatively poor predictor of spatial patterns of industrial activity, so this option should only be used if no additional information is available. In the third scenario, additional data provided by the IA method developer is used to estimate how much of the inventory activity occurs in each IA spatial unit. For example, an IA method with a spatial scale of watersheds probably has background data on withdrawals from each watershed, while a new method developed specifically for industry may use the aggregate of regional industry data. We have developed spatial extension matrices, which allow new data sources on a third spatial scale to estimate the spatial pattern of inventory data. This approach requires additional data collection, but is quite powerful. Our matrix formulations have a number of advantages. First, they allow re-projection of regionalized LCA results to each spatial scale, making visualization and interpretation easier. Secondly, while GIS calculations are required for matching for only one step once for each method and inventory database, meaning that LCA software can use pre-calculated values instead of integrating GIS logic. [1] https://brightway2-regional.readthedocs.org/en/latest/

MO380 Application of the LC-IMPACT impact assessment method to ecoinvent
C. Mutel, ETH Zurich / Laboratory for Energy Systems Analysis; F. Verones, NTNU / Department of Energy and Process Engineering; K. Volkart, Paul Scherrer Institut / Laboratory for Energy Systems Analysis; S. Hellweg, ETH Zurich / Institute of Environmental Engineering

LC-IMPACT is a new, comprehensive and global impact assessment method that provides spatially differentiated characterization factors for water use, land use, terrestrial acidification, freshwater and marine eutrophication, toxicity, and particulate matter. Regionalized characterization factors are available in a standardized format. We describe how to systematically apply LC-IMPACT to the ecoinvent inventory database. The methodology for regionalized impact assessment is new and under development, and the key area of difficulty when combining inventory databases and impact assessment methods at appropriate spatial scales is the choice of spatial allocation weights [1]. A naive approach is to use the relative intersected areas of each inventory and impact assessment spatial unit. However, this approach produces incorrect results when inventory activities are not uniformly distributed in space. Additional data, which we call extension matrices, can be used to approximate the values of the interfacing inventory activities that would be industry-sector specific. We illustrate this concept with global maps of agricultural activity and GDP-weighted population density. The ecoinvent inventory database provides detailed spatial data for each inventory process [2]. However, the location “Rest of the World” is widespread – almost one quarter of all processes in ecoinvent 3.1 - but this location has no provided spatial definition, but rather is defined dynamically as whatever isn’t covered by more specific datasets.

MO381 Carrying capacity based normalization references at the endpoint level
A. Bjorn, Technical University of Denmark / Department of Management Engineering; C. Bille, CIRAIQ-G ESG - UQAM / Strategy corporate social responsibility; M. Margni, Ecole Polytechnique de Montreal / Mathematical and Industrial engineering; M. Hauschild, DTU Management Engineering

The popularity of the ecological footprint method and the planetary boundaries concept shows an increasing interest among decision makers in comparing environmental impacts to carrying capacities of natural systems. Recently carrying capacity based normalization references were developed for impact categories at the midpoint level in LCA. Building on this work we propose two sets of global average normalisation references compatible with the endpoint categories compiled in the Impact World+ methodology. The first set of references was based on a translation of the science-based thresholds for which the midpoint normalization references were based, combined with the use of additional thresholds for impact categories that are covered by those references. The second set of references was based on defining carrying capacity as a consistent level of species protection at endpoint, here HCS(NOEC), the concentration at which maximum 5% of species are affected above their no observed effect level. The resulting endpoint normalisation factors were together with the existing references at midpoint applied to a life cycle inventory of food consumption in the Netherlands. The resulting ranking of normalised impact scores for the different impact categories was compared for the three approaches to carrying capacity-based normalisation. The ranking was found to differ considerably between the approaches with the implication that the results only partly agreed on how to prioritise impact reduction efforts of Dutch food consumption. Moreover large differences between the absolute values of normalised impacts were found between the three approaches for some impact categories, meaning that Danish food consumption may appear environmentally sustainable when using one set of normalisation references and unsustainable when using another set of references. This work expands the applicability of carrying capacity-based normalisation and furthermore shows the influence of value choices in the quantification of carrying capacity.

MO382 Recommended impact assessment methods for ILCD and Environmental Footprint:
challenges, opportunities and updates
S. Sala, European Commission DG Joint Research Centre / Institute for Environment and Sustainability; L. Benin, IRC for an update of the recommendations both from a harmonisation perspective, as well as for scientific credibility. In the context of the PEF, the EC-JRC is in the process of updating ILCD recommendations, focusing mainly on 4 impact categories, namely: water depletion (WD), resource depletion (RD), land use (LU) and respiratory inorganic (RI). In order to support an update of the ILCD and PEF recommendations a three-step evaluation procedure has been put in place: i) qualitative screening for newly developed methods, ii) benchmark among the available methods; iii) applicability analysis wit reference to life-cycle inventory data and elementary flows. This approach will ensure identifying not only the (theoretically/scientifically) best LCIA method but also whether there any significant gaps in LCIA data availability to feed the LCIA models or issues regarding unexpected results and anomalies that need further attention. The limits of the three-step procedure will be extrapolated in the near future and some of the results of the screening are provided. As regards the LCI, it seems reasonable to expand the ILCD method, at least at mid-point level, accounting more than one impact category, considering the multi-functionality of land. The selection of method(s) was also based on the availability of scientific literature and case-studies, and in order to facilitate the applicability with the currently available datasets containing inventory data on LU. Concerning WU, special attention was given to aspects such as consumptive use only vs. overall withdrawals, spatial differentiation, temporal scale environmental water requirements, as well as the applicability of the methods to currently available datasets. In case of RD, the three-step evaluation procedure is preceded by a careful consideration of what should be assessed in terms of impact on the Area of Protection Natural Resources (AoP), given the poor substantiation of this AoP in the LCA community so far.

MO383 Coupling nutritional and environmental aspects of food and beverage: how could it be tackled?

Environmental sustainability of food is one of the most relevant issues within the sustainable development context. Since the primary scope of food is nutrition, environmental aspects should be assessed adopting an integrated approach with nutritional ones. In order to tackle this cycle, in the present study a multi-criteria indicator coupling nutritional quality and environmental impacts

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categories is developed. After a thorough survey of the scientific literature, a certain number of food and beverage products belonging to different food groups (i.e., proteins, vitamins, carbohydrates and beverage) were selected. Each one of the selected products was characterized in terms of both environmental and nutritional terms. At first instance, only two indicators are taken into account concerning environmental dimension: carbon footprint (adopting characterization factor estimated by IPCC, 2013) and water footprint, calculated following the recently published ISO 14046. The nutritional quality of food and beverage in addition is added through the use of three indicators: the Fullness Factor indicator, the Nutrient Rich Food Index and the Calories for Nutrient. The Fullness Factor (developed based on data provided by USDA’s National Nutrient Database for Standard Reference) is a dimensionless index which tries to quantify the satiety of a food or recipe compared in a nutritional and environmental aspect. Silva, ESALQ; S. Drogue, INRA / UMR MOISA; G. Ferrari, INRA; N. Kaplin, Outokumpu Stainless AB; M. Hauschild, DTU Management Engineering; A. Ometto, Universidade de Sao Paulo / Departamento de Engenharia de Produção Escola de Engenharia de Sao Carlos; M. Hauschild, DTU Management Engineering Brazil is the largest country in Latin America. Its extensive geographical area, with important variations in population density and environmental properties makes it difficult to properly assess environmental impacts using site-specific characterization factors. The application of spatially-differentiated Life Cycle Impact Assessment (LCIA) methods could better capture the differences of climate, topography, soil, fauna, flora and diverse activities of each region in Brazil. In this context this work aims to provide a first step towards spatially-resolved characterization factors for acidification, eutrophication and photochemical ozone formation in Brazil. Eutrophication factors (FF) for airborne pollutants, i.e. nitrogen oxides, sulfur oxides, ammonia and non-methane volatile organic compounds (NMVOC), contributing to these impacts were thus determined from source-receptor matrices (SRMs) based on simulations at a 2° x 2.5° grid resolution. The simulations were performed by GEOS-Chem v9-02 model, which was assessed as the most comprehensive and robust model for the California School of Public Health; R.K. Rosenbaum, National Research Institute of Science and Technology for Environment and Agriculture - Irstea / UMR ITAP; D. van de Meent, RIVM / DMG; M. Hauschild, DTU Management Engineering USEtox is the UNEP/SETAC scientific consensus model for assessing human toxicological and ecotoxicological impacts of chemical emissions. The model is being widely used in comparative toxicity assessments and officially endorsed by the UNEP/SETAC Life Cycle Initiative since May 2013. We present the next official release version, USEtox 2.0. The new USEtox 2.0 provides midpoint and endpoint characterization factors for human toxicity and freshwater ecotoxicity for more than 4000 organic and inorganic chemicals. Major improvements and updates compared with the previous version include: (a) the following updates: (a) ionizing organic chemicals, (b) residential and occupational indoor environments, (c) exposure to pesticide residues via food crop consumption, (d) generic freshwater ecotoxicity for metals, (e) 900+ new substances and updated substance data, (f) continent- and sub-continent-specific landscape parameters, and (g) a user-friendly and intuitive user interface. With these updates, USEtox contributes to addressing the high demand for improving the characterization of toxic chemical emissions and is readily operational for use in life cycle assessment, footprinting and other comparative assessments. The new USEtox 2.0 model and factors can be exclusively accessed free of charge at http://usetox.org.
typically consisting of 18 wt% chromium, 8 wt% nickel and 74 wt% iron. When the life cycle performance of alloy products and metals processing is described in life cycle assessment (LCA), several sources for errors exist. The research presented here aims to investigate whether the modelling of metal runoff from stainless steel surfaces is an important factor for its life cycle performance. A stainless steel roof is chosen as a case study in which two ways of modelling of metal runoff is excercised: (1) by calculation on the individual alloy components, and (2) based on measured long-term runoff data (4 years) from the alloy at field conditions. A life cycle assessment of a stainless steel roof was performed for a base case and an alternative case. The use phase was in the base case modelled with metal runoff data based on experimental findings for the main individual alloy components (Fe, Cr, Ni), and in the alternative case with runoff data based on long-term metal runoff measurements at urban sites. By comparing the 304 stainless steel roof runoff data showed to have a great importance for the end result. The results from the case study also compared the order of magnitude of the modelling of metal runoff as an error source, and compared this with two other error sources: the LCIA and the data inventory of the metal production. All three error sources showed to have large influence on the end result, but that the metal runoff data was the error source of largest importance. We therefore suggest that real observed metal runoff data will be included in future LCA studies of metals and alloys.

MO389  
Integration of the metal bioavailability in freshwater for the ecotoxicity footprinting with the USEtoxTM model database  
J. Juge, CIRAIG; O. Huguenot, Toolenv; T. Pavigy, Cycleco  
Keywords: Bioavailability factor, metals, USEtoxTM model Many pollutants are found in the environment with several physicochemical properties which provide different behaviours. Metals behaviour differs from organic compounds: they are infinitely persistent, octanol-water partition doesn’t predict their tendency to bioaccumulate and only the truly dissolved fraction is considered to be bioavailable. For the reason of that reason, physicochemical properties of metals in the USEtoxTM model differs from organic compounds. The USEtoxTM model was developed under the UNEP-SETAC Life Cycle Initiative, by a team of researchers, and provides the state-of-the-art for the ecotoxicological impact assessment of substances. Conscious of the specificity of metals and the need to take into account bioavailability to avoid overestimation, we wished to update the characterization factors (CF) available in the ecotoxicological footprinting database using the USEtoxTM model. Dong et al. (2014) publication provided CF for 15 cationic metals. The $K_{p}$ and the $K_{DOC}$ were calculated with the model WHAM 7.0 which takes into account the equilibrium-based metal speciation model, for a specific water chemistry. Physicochemical parameters and Effect factors (EF) from Dong et al. were implemented preferentially. If not available, the EF came from the AiiDA database and the physicochemical parameters came from the USEtoxTM inorganic database, from Echachem database or finally estimated by calculation. The landscape was changed to approach a representative European freshwater river and physicochemical parameters of metals were selected for this same river. Updates relate to the 15 cations from the Dong et al. publication and the seven cations from the USEtox database. Changing $K_{p}$s and $K_{DOC}$s found by Dong et al. in the ecotoxicological footprinting database allows to find the same bioavailability factor (BF). This shows that other physicochemical parameters implemented before this work don’t have a significant impact on this factor. The new CF for the freshwater range from 9.6E6 (Fe III) to 1.1E1 (Cd II). From now on the ecotoxicological footprinting with the USEtoxTM model database, the bioavailability of metals is better quantified. This is now available in the online database for 22 cations. Up to now, BF existed in recent publications but had not been yet implemented in models to calculate CF.

MO390 Including zinc speciation in LCA terrestrial ecotoxicity: does it make a difference for LCA results?  
Metals are often the main contributors to ecotoxicological impacts in life cycle assessments (LCA). Metals are typically treated as one single entity, without addressing the importance of metal speciation in terrestrial ecotoxicity in LCA. A case study on the ecoinvent market for electricity, low voltage CA-QC data was carried out using the IMPACT 2002+ methodology. Results obtained using present terrestrial ecotoxicity characterization factors (CFs) from IMPACT 2002 were compared to results obtained using new regionalized CFs that make zinc speciation into account. These CFs were obtained by using modified USEtox fate factors, WHAM 6.0 derived bioavailable factors and effect factors calculated using the AMI method with available terrestrial ecotoxicity data. Generic default CF values for the world based on either soluable or true solution (free ion and ion pairs) Zn as well as minimum and maximum regionalized CF values were tested in this study. Results indicate that for this specific case study, the contribution of Zn emitted to soil to the terrestrial ecotoxicity impact score falls from 26% to a maximum of 1.27%, impact scores being reduced by 1.44 to 14.37 orders of magnitude, when Zn speciation is taken into account. Also, when including Zn speciation in terrestrial ecotoxicity, the total terrestrial ecotoxicity and ecosystem quality impact scores decrease by approximately 25% and 21%, respectively, and among the major contributors to terrestrial ecotoxicity, Zn falls from the 2nd to being the 9th and 255th position. Results show that integrating Zn speciation into the CF definition highlight the significance and feasibility of accounting for metal speciation in soil in an LCA context.

MO391 TOX-TRAIN: the user-friendly toolbox for human and ecotoxicity assessment  
X. Bengou; M. Birkved, Technical University of Denmark; P. Fantke. Technical University of Denmark / Quantitative Sustainability Assessment; S. Humbert, Quantis; S. Sourisseau, VEOLIA Environnement Recherche et Innovation; R. Van Zelm, Radboud University  
It is estimated that it is 10 to 20 thousand different chemicals are regularly used in the life cycle of products, many of which can potentially have harmful effects on humans or ecosystems. Industries are more and more interested in understanding the toxicity of their products constituents and are willing to act on their supply chain to reduce the amount of dangerous substances emitted through all stages, from manufacturing and processing to end-of-life. The four-year EU-funded TOX-TRAIN project aims at creating a user-friendly toolbox based on the scientific consensus model USEtox® and promoting this model, in order to assess human toxicological and ecotoxicological impacts related to emissions over the life-cycle of technologies. Developed under the supervision of the Life Cycle Initiative lead by the UNEP-SETAC, USEtox® assesses such impacts for numerous chemical substances®. The main output of TOX-TRAIN will be a toolbox that combines the official USEtox® model with additional assessment tools. In a user-friendly interface and with full documentation, TOX-TRAIN has already achieved several of its objectives. A thorough literature review of existing chemical inventory models was made, which led to the development of the sewage emission model SewageLCI®. This model is based on a set of chemical and national input parameters capable of quantifying the fraction of a chemical emitted to sewage systems ending up being subsequently released to the individual USEtox® emission compartments. Exposure models for household, workers, and direct exposure were developed and will constitute additional modules of the TOX-TRAIN toolbox. Characterization factors for persistent bioaccumulating chemicals, persistent active surface chemical compounds, pesticides and biocides have been developed and their uncertainty quantified®. All these developments will be tested and evaluated in cases studies. Finally, several courses and workshops have been organized at different venues to inform and train on USEtox® and its application within the LCA framework. Outputs and development from TOX-TRAIN will all become publicly available and will be evaluated for future integration into USEtox®.

LCA of complex systems: delusion or reality? (P)  
MO392 Integration of simple LCA into complex systems: the CaSIE² project  
E. Lens, ODME, CODDE; J. Orgelet, Bureau Veritas CODDE / Ecolife; P. Fantke, Technical University of Denmark; P. Fantke, Technical University of Denmark / Quantitative Sustainability Assessment; R. Van Zelm, Radboud University  
Integration of simple LCA into complex systems is a long and fastidious task. In order to allow a better diffusion of the LCA methodology a long and fastidious task. In order to answer this need. This study will be supported by the ELODIE and EIME projects. The research presented here aims to investigate whether the modelling of metal runoff from stainless steel roofs is performed for a base case and an alternative case. The use phase was in the case study modelled with metal runoff data based on experimental findings for the main individual alloy components (Fe, Cr, Ni), and in the alternative case with runoff data based on long-term metal runoff measurements at urban sites. By comparing the 304 stainless steel roof runoff data showed to have a great importance for the end result. The results from the case study also compared the order of magnitude of the modelling of metal runoff as an error source, and compared this with two other error sources: the LCIA and the data inventory of the metal production. All three error sources showed to have large influence on the end result, but that the metal runoff data was the error source of largest importance. We therefore suggest that real observed metal runoff data will be included in future LCA studies of metals and alloys.
MO393 Life Cycle approaches for holistic assessment redevelopment urban projects (brownfields)
C. Hidalgo, Leitit Technological Center / RD Safety - Sustainability Division; M. Escamilla, LEITAT / Sustainability Unit; J. Boronat, L. Nadal, Medi terra; G. Louwagie, European Environment Agency
In many cases there are abandoned or under-used industrial areas, which were developed previously and can have residual in-situ contamination from earlier activities. These areas, also called brownfields, can be remodelled in order to satisfy the increasing demand for new urbanized areas with different purposes, including residential, infrastructure or green areas. Urban planning actuators have associated environmental impacts over a long time period, as the result of planning conditions the behaviour of cities over several years. From a sustainability point of view considering all life cycle stages, including early stages, is desirable. Holistic approaches supported by comprehensive tools are needed to guarantee this view. Existing literature suggests that the environmental impacts of brownfields projects are not considered in a holistic manner. Usually only specific aspects such as selected impacts from remediation or construction activities are assessed. Therefore, applying the Life Cycle Assessment (LCA) approach to brownfield developments is seen as a good opportunity to assess their overall environmental profile and identify possible management improvements. Recently EEA has led a study to explore the feasibility of applying LCA approach to reusing brownfields as part of urban planning. Three case studies were analysed including two brownfields and one greenfield area. The study shows the potentiality of applying fully life cycle thinking approaches to brownfields and urban (re)development projects. The selection of a representative functional unit (depending on the planned use, the surface, built form, or the number of residents/users) proved to be a key parameter affecting the results. The findings show that, for the three cases, the most relevant life stages in terms of environmental impacts are the use stage (with a duration of 20 years), as well as the development stage with important contributions from the construction of new buildings and construction works.

MO394 Where to look for the complexity of EcoInvent v3 process networks?
T. NAVARRETE GUTIERREZ, CRTE; E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE; A. Marvuglia, Luxembourg Institute of Science and Technology (LIST) / Resource Centre for Environmental Technologies CRTE; R. Bugani, Centre de Recherche Public Henri Tudor (CRP Henri Tudor) / Centre de Ressources des Technologies pour l’Environnement CRTE; E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE
The latest EcoInvent database (version 3.1) includes several new features like updated and new datasets, and more system models. As the database improves in quality and features, one could assume that its representation of the complex real world is also improved. One way to confirm or deny this assumption is by looking at the structure of the process network of the database and evaluate its degree of complexity, which is compared to other well-known networks (that is, the ones they exhibit non-trivial characteristics absent in random networks). With this in mind, we shall present a comparison of the graph obtained from the EcoInvent version 2 database and the one of version 3. We are interested in measuring the graph following traditional network analysis methods to characterize each network. The measures include determining the importance of a node, in regard to the number of paths that go through it, or the number of neighbours, as well as the diameter of the network. Creating such graphs allows to compare both graphs (EcoInvent v2 and v3) against each other, but also against well equivalent complex networks of the small-world family, random networks, and scale-free networks (of the Barabasi-Albert family). Different libraries for network analysis, including NetworkX and igraph, scripted through the Python language. Also, we used implemented different algorithms present in the literature using R. Finally, gephi was used for creating the figures depicting the graph structure. Preliminary observations suggest that although the EcoInvent networks are definitely not random, they exhibit some characteristics also present in scale-free networks (power law distribution of the cumulative degree distribution), but not those of small-world networks (short average path length, high clustering coefficient).

MO395 Life Cycle Assessment of Power-to-Gas for Integrated Energy System Analysis
X. Zhang, Laboratory for Energy Systems Analysis; C. Mutel, Paul Scherrer Institute / Laboratory for Energy Systems Analysis; C. Bauer, Group Environment, Health & Safety
The future Swiss electricity supply system is expected to rely strongly on stochastic renewable generation such as photovoltaic and wind power. As a consequence, more and more flexibility is required and storage technologies and conversion of different types of energy carriers will play a vital role in the integrated energy systems of the future. As part of the recently established “SCCER Strategies for Energy Research Centre” two projects aim to assess the technologies will be performed to better understand the impacts of these technologies in terms of environment, economy and their technology performance, and how they could be integrated into the Swiss energy system in the future. Life Cycle Assessment (LCA), as a key component in the technology assessment framework, has been applied to investigate the environmental impact of these technologies. In this poster, an individual how LCA, which is not a technology assessment (similar to the more conventional scenario approach). Additionally, LCA aims at assessing the dissemination of LCA to policy makers through a collaborative approach and by developing tools allowing policy makers to interact with the model, influencing the policies during the course of the simulation and visualizing the results.

MO396 Life Cycle Assessment of Power-to-Gas for Integrated Energy System Analysis
A.L. MERCHAN, University of Liege / Applied Chemistry
The European Commission’s White Paper on transport [1] seeks to achieve an efficient and sustainable balance between the various transport modes. In this context, BRAIN-TRANS is a project supported by Belgian Federal Government that deals with rail freight intermodality, approaching the problem from an interdisciplinary perspective. BRAIN-TRANS will be able to answer the transition involved in transportation in Belgium. Environmental impact studies on intermodal transport show that rail freight transport in the land-based transport that has a higher environmental performance (more fuel-efficient shipping alternative) compared to intermodal road-rail and all-road transport [2]. This is especially the case when electrified railway is used. But there is not always a direct rail link, so the intermodal road-rail transport is generally shown as the best alternative. The increased demand for rail transportation raises the need for the expansion of the railroad system and the environmental effects that infrastructure construction entails should be taken into account. Life Cycle Assessment (LCA) methodology allows us to model as best as possible the environmental impacts of several pollutants in numerous categories: CO2, NOx, SOx, NMVOC, particles, and CO2 emissions. For other categories such as accident damages, noise impact and passenger cars, which heavily rely on the internal combustion engine, are the source of several environmental burdens, from contribution to climate change to urban air pollution. To reduce its environmental impact, sustainable transport policy focuses on promoting the use of less pollutant alternative means of transport (e.g. bicycle, train, bus) and the development of “green” vehicles (e.g. hybrid or electric vehicles). Attributional life cycle assessment (LCA) usually aims at assessing either the environmental impacts of one means of transport, comparing two, using simplifying generic hypotheses. In CONNECTING, we aim at assessing the impacts at the policy level, which could be for instance the installation of charging infrastructure at railway station public parking, decreasing train ticket prices. For this purpose, we develop an agent-based model (ABM), which simulates a population of commuters in the cross border area of Luxembourg taking various transportation means to go to work. These commuters, represented in the model by agents who have their own characteristics (purchase power, mobility needs, personal behaviour, etc.), can interact with each other and react to external stimuli. This ABM is fed by numerous data at both macro-level (e.g. national statistics) and micro-level (e.g. individual surveys). The ABM is then coupled with an LCA model, which calculates the environmental impacts of the agents. The model is first run without a specific policy and the sum of the environmental impacts corresponds to the reference situation. Then, the policies under study are applied to the model and their consequences are evaluated as the difference between the results obtained and the reference. The ABM-LCA coupling differs from traditional consequential LCA, where scenarios or economic macro-modelling are used to derive inventories. In this project, the impact of the policies is the result of the interactions of a large number of individuals (agents), which are not predicted, but rather decisions of free networks.

A Probabilistic Fuzzy Multiojective Linear Programming Model to Design Reverse Logistics Networks Based on Life Cycle Assessment

W. Ocampo-Duque, L.I. Duque, Pontificia Universidad Javeriana Cali / Industrial Engineering; O.O. Ortiz, Universidad de Panplona / Industrial Engineering; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering

Determining the optimal distribution network for raw materials is a problem of great economical and environmental importance. The goal of the study was to determine the optimal network that minimizes environmental impacts and costs associated with the management of used tires produced in a Colombian region. The proposed network included diverse activities such as picking, selection, and cleaning. Different configurations of the logistic network were assessed by varying the percentages of product devoted to primary alternatives. The configuration with optimal solutions was generated. The proposed network provided a feasible and economically sustainable alternative to solve the complex problem of used tires management.

A. Bouter

MO398
Life Cycle Assessment of territorial biogas production by anaerobic digestion of local wastes

A. Avadí, INRA-Agrocampus Ouest / UMR SAS; M. Conson, INRA Institut National de la Recherche Agronomique

Life cycle assessment (LCA) is rarely used to estimate environmental impacts of regions, given its product-level scope and costs of analysing all processes in a region. Nonetheless, one can analyze individual samples (e.g. farms) and extrapolate the analysis to describe a population (e.g. all regional agricultural activity). Linear extrapolation is often used to extrapolate farm-level impacts, but it is more accurate to do so as part of an analytical approach (e.g. a region as an aggregation of farm activities) and also to acknowledge that these approaches infrequently consider factors such as spatiotemporal explicits, intra-regional exchanges, and farm variability. We applied an LCA extrapolation approach to the agricultural Lieue de Grève catchment in Brittany, France. To estimate regional impacts, we first performed LCA of 25 farms in the region. In parallel, we constructed a farm typology using principal component analysis and clustering of all farms in the region with sufficient structural and operational data (114 farms: 34% of the catchment area). We ensured that each cluster included a minimum number of farms with LCA results and that their impacts per ha (for selected categories) were within ±25% of each other. Next, using multiple linear regression (MLR), we estimated environmental impacts for all farms in the largest cluster (dairy farms) as a function of a set of input predictors. For this cluster, we included LCA results from other Breton dairy farms in the MLR so that the sample size was sufficiently large given the number of predictors in the best-subset regression of the MLR. We then calculated mean impacts from both LCA and MLR of all farms in each cluster and included corrections for intra-regional exchanges (balancing imports and exports of fodder and manure for all farms to determine whether the average farm had a deficit or surplus). Finally, each cluster’s mean impacts were multiplied by the total area of each cluster in the region, and the impacts of centralised regional activities (e.g. communal fodder drying) were added to the extrapolated results. Conceptually, by taking characteristics of more farms in the studied region into account, this approach should estimate environmental impacts of all of its agricultural land more accurately than linear extrapolation. The approach is being used to compare regional impacts before and after a series of agricultural innovations.

Bioavailability of organic chemicals: Linking science to risk assessment and regulation (P)

A. Avadí, INRA-Agrocampus Ouest / UMR SAS; M. Conson, INRA Institut National de la Recherche Agronomique


Bioavailability of PAHs in Fuel Soot Assessed by an in vitro Gastrointestinal Model Including a Third-phase Absorptive Sink: Effect of Food Ingestion on PAHs

M. Duque, L.I. Duque, Pontificia Universidad Javeriana Cali / Industrial Engineering; A. Rodella, Rovira i Virgili University / Chemical Engineering

The aim of this study was to develop an in vitro gastrointestinal absorption model of polycyclic aromatic hydrocarbons (PAHs) and determine the extent to which food ingestion could affect intestinal absorption of PAHs in fuel soot. The model included a third phase, representing food in the gut, in which PAHs adsorbed to charcoal as an absorptive sink. The model was validated using the in vivo gastrointestinal absorption model of PAHs in fuel soot from a previous study. The in vitro model predicted absorption well, with a correlation coefficient of 0.87. This suggests that food ingestion could affect intestinal absorption of PAHs in fuel soot.

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Bioavailability of organic chemicals: Linking science to risk assessment and regulation (P)

A. Avadí, INRA-Agrocampus Ouest / UMR SAS; M. Conson, INRA Institut National de la Recherche Agronomique

of unknown substances and has the advantage that each unique discharge is tested, rather than relying on predictive field toxicity values. Whole Effluent Assessment (WEA) was carried out on 15 platforms in the UK Continental Shelf as part of an assessment of the applicability of WEA as part of a risk based approach for the monitoring of produced water in the offshore industry. Installations were chosen to give a broad coverage of location, type of production (oil, gas), stage of production (early, late) and characteristics of produced water (oil content, water cut, use of chemical) to reflect the variability of produced water based on previous biannual data submitted. The UK study was carried out in parallel with other studies in Norway, The Netherlands and Denmark. WEA testing was carried out using bioassays from three trophic levels: bacteria (MARA and LumiMARA), algae (Skeletonema costatum) and crustacean (Acartia tonsa). WEA testing was carried out on produced water samples collected offshore at the same sampling point/time as samples were collected for biannual chemical sampling. Samples were shipped onboard as quickly as possible for analysis. Samples were aged at 15°C for three weeks and WEA testing repeated. For six of the installations bacterial WEA was carried out with MARA and LumiMARA offshore at the point of sampling. Biodegradation (OECD 306) was carried out onboard on all 15 installations.

MO404
Environmental Effects of Triclosan
A. Peters, D. Leverett, WCA-Environment Ltd
Triclosan is an antibacterial agent which is added to a wide range of household and personal care products. Its use in such products means that the primary route of release to the environment is via the manufacturing process. In systems the reactions to form the grease thickener occur during the grease manufacturing process and consequently these grease thickeners generally only exist in the presence of the “blended” base oil matrix. Most greases are based on the use of metal-soap, metal-complex-soap or polyurea (PU) grease thickening agent systems. In some circumstances it is not practically feasible to generate test data for grease thickeners as they are found in many natural surface waters. A significant limitation of predictive models is the need to consider the toxicity of different substances under high pH conditions, this implies that there will be consistent differences in the sensitivity of different surface waters due to differences in local pH conditions. If the SSD is not normalised to a single pH value then there will always be some remaining uncertainty as to whether or not the resulting thresholds are likely to be protective of the most sensitive water chemistry conditions. An assessment of the potential influence of pH on triclosan toxicity, and the sensitivity of surface waters to triclosan are presented.

MO405
Evaluation of bioavailability and toxicity of grease thickeners using SPME
G.F. Whale, Shell Health; Shell Health; C.V. Eadsforth, Shell International; T. Corry, Shell Health
Grease thickeners are an intrinsic part of greases and are often formed in the manufacture of greases and soap, during the grease manufacturing process and consequently these grease thickeners generally only exist in the presence of the “blended” base oil matrix. Most greases are based on the use of metal-soap, metal-complex-soap or polyurea (PU) grease thickening agent systems. In some circumstances it is not practically feasible to generate test data for grease thickeners as they are found in many natural surface waters. A significant limitation of predictive models is the need to consider the toxicity of different substances under high pH conditions, this implies that there will be consistent differences in the sensitivity of different surface waters due to differences in local pH conditions. If the SSD is not normalised to a single pH value then there will always be some remaining uncertainty as to whether or not the resulting thresholds are likely to be protective of the most sensitive water chemistry conditions. An assessment of the potential influence of pH on triclosan toxicity, and the sensitivity of surface waters to triclosan are presented.

MO406
On the challenge to assess the exposure of organic contaminants in matrices with high partition coefficients
I. Hilber, Agroscope ART; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; V. Gouliamou, Aarhus University, Science and Technology Faculty / Environmental Chemistry and Microbiology; S.E. Hale, Norwegian Geotechnical Institute / Environmental Engineering; G. Cornelissen, NGL. T.D. Bucheli, Agroscope ART / Analytical Chemistry Natural Resources Environmental Protection in Agriculture
To assess aqueous concentration (Caq) and bioaccessibility of organic contaminants in matrices that have high solid-to-water phase partition coefficients (log Kow > 5) is an inherent challenge. Experiments that achieve efficient mass transfer and infinite sink conditions need to be properly designed. One key parameter controlling the (de) sorption process is the magnitude of the Koc value of active compounds in the sample and the sink, and Koc values, multiplied by the massfraction polymer over the mass fraction sample, should be at least 10 for depletive methods (bioaccessibility) and < 0.01 for non-depletive methods to determine Caq of a pollutant. We have chosen to investigate biochar as challenging matrix due to its PAHs formed during pyrolysis, the expected high Koc and its allotegemor and hygroscopic nature. The slowly pyrolysed elephant grass (biochar 1) and sieved coniferous wood residues (biochar 2) had total concentrations of the sum (>16 US EPA PAHs of 63 mg/kgDw 355 mg/kgDw, respectively. Polyoxyethylene was equilibrated with the biochars to assess the Caq [1], whereas Tenax [2], silicone rods [3] and activated carbon/silicone composite (1.9 mass ratio) [4] were used as sorptive sink for the determination of PAH bioaccessibility. Cyclodextrin was in some of the approaches used as diffusive carrier [3, 4] in order to enhance the PAH desorption from the biochar. The Caq of >16 US EPA PAHs was 52 ng/g for biochar 1 and 71 ng/L for biochar 2, which are very low levels for a matrix containing PAHs in the mg/kg range. The reason for these low Caq values was the very high log Koc values that ranged from 5.7 for naphthalene in biochar 1 to 7.6 for pyrene in biochar 2. The results from the depletive methods revealed that not all of 1-2 substances in each category were measured accurately and conservatively assess the exposure and the risk and thus facilitate regulatory acceptance. Future research should address the influence of biochar stability.

MO407
Comparing modelled ecotoxicity predictions using PETROTOX and SPME data on petroleum products to screen for aquatic toxicity
C.V. Eadsforth, Shell International; M. Comber, ExxonMobil Petroleum and Chemicals; S. Linington, BP Castrol Technology Centre; K. den Haan, CONCAWE / Petroleum Products Safety; A.D. Redman, Exxon Mobil Biomedical Sciences; J. Butler, ExxonMobil Biomedical Sciences, Inc / Environmental Toxicology and Chemistry Laboratory; D. Lentinis, ExxonMobil Biomedical Sciences
Using detailed two-dimensional chromatography (GCxGC) analysis of a set of petroleum product samples of Gas Oils, Residual Aromatic Extracts (RAE) and Bitumen categories, predictions using PETROTOX have been used to provide information to support revised category justification documents and to enable the selection of the most suitable substances in each category for ecotoxicity testing. In addition, analysis of Water Accommodated Fractions (WAFs) of these product samples using Biomimetic Extraction (BE) with solid phase microextraction (SPME) fibres was used to confirm that (a) SPME data correlates to Toxic Units predicted by the PETROTOX model using GCxGC compositional data as input and (b) the linkage between composition, SPME data and aquatic toxicity is strengthened. This provides a technical basis for further use of SPME as a more practical characterization tool for addressing the influence of variation in substance composition on aquatic toxicity within petroleum product categories. BE-SPME is therefore shown to be a cost-effective approach to toxicity screening for petroleum substances, thereby offering an alternative method to enhance the current available ecotoxicity data sets, as well as being complementary to predicting ecotoxicity using PETROTOX.

MO408
Relating Bioavailability Parameters to the Sorbent Characteristics of PAH Polluted Soils
N. Goulamou, Agroscope Reckenholz-Tänikon Research Station ART / Analytical Chemistry Natural Resources Environmental Protection in Agriculture; I. Hilber, Agroscope ART; R. Schulin, ETH Zurich; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; T.D. Bucheli, Agroscope ART / Analytical Chemistry Natural Resources Environmental Protection in Agriculture
Reduction of Hydrophobic Organic Contaminants (HOC) such as polycyclic aromatic hydrocarbons (PAHs) in soil is still based on total concentrations. However, many studies have demonstrated that not all of a pollutant’s content in soil is equally available to organisms (Reichenberg & Mayer 2006). Over the last decade, intensive effort has been made to incorporate bioavailability into risk assessment (Cachada et al. 2014). Here, we compare total concentrations of PAH with two bioavailability parameters in 30 different soil samples from the archive of the standardized National and Zurich Cantonal Swiss Soil Monitoring Network (NABO and KABO). The selected samples were chosen to cover a wide range of total PAH concentrations, sources of origin, and soil types. We applied a depletive method with silicon rods for measuring bioaccessibility (Goulamou & Mayer, 2006).
Microbial motility: a behavioural approach for bioassays in toxicity assessment

C. Jimenez Sanchez, Inst de Recursos Naturales y Agrobiol. de Sevilla / Agroquimica y Conservacion de Suelos; R. Sunghong, Instituto de Recursos Naturales y Agrobiologia de Sevilla. CSIC / Agroquimica y Conservacion de Suelos; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiología / Agroquímica y Conservación de Suelos

The biological assessment of pollutant toxicity rarely includes behavioural responses. In this work, we aim to introduce the swimming behaviours and tactic responses of motile microbes for assessing the toxicity of pollutants (nanoparticles (NPs) and polycyclic aromatic hydrocarbons (PAHs)). Two different microbes were used in this work, i) a naphthalene-degrader bacterium, Pseudomonas putida G7, and ii) swimming algae of the thallophyta oomycete (lower fungus) Pythium anphanidermatum. A computer motion analysis software (CellTrak) was used for evaluation of microbial swimming behaviours, and a chemical-in-capillary method in connection with microscopic observations and viability tests were used for evaluation of microbial tactic responses towards the pollutants. We observed that both silver and iron NPs influenced the swimming patterns of P. putida G7 by increasing tortuosity motility. Both NPs caused a repellent effect only at a specific range of concentrations, but iron NPs showed a greater repellece possibly due to its higher toxicity to P. putida G7. We found also that PAHs (naphthalene and phenanthrene) influenced the tactic responses of zoospores towards a set of chemical effectors that have relevance for bio remediation. It was suggested that bioavailability of PAHs was a key factor for their toxicity effects on zoospore taxis. With these results, a petition of a unique set of motile microbes could be a useful model to evaluate the toxicity of pollutants at environmental relevant concentrations. In addition, the role of behavioural responses on actual pollutant exposure concentrations should be taken into account for a realistic assessment of pollutant toxicity at the microscale.

MO412 Aged spiked soils cannot resemble desorption and bioaccessibility of native PAHs in historically contaminated soils

A.P. Lohbner, Boku, IFA-Tulln / Department for Agrobiotechnology IFA-Tulln; K.E. Scherr, University of Natural Resources and Life Sciences, Vienna / IFA-Tulln; E. Edelmann, University of Natural Resources and Applied Life Sciences / Agrobiotechnology- IFA Tulln; S. Humel, D. Kopp, BOKU / Department for Agrobiotechnology IFA-Tulln; P. Mayer, Technical University of Denmark / Department of Environmental Engineering

In the present study, 25 Austrian soils were collected and spiked with four selected polycyclic aromatic compounds. Using the contaminant trap, PAH desorption between freshly contaminated and aged/field PAH spiked soils was compared. A sampling of three historically PAH-containing soils. The observed differences could not be explained by physical entrapment of PAHs in historically contaminated soils since grinding of these soils did not enhance PAH desorption from the soils. The addition of high amounts of toluene to historically contaminated soils resulted in enhanced PAH desorption and a lower desorption resistant fraction. This observation is in line with competitive binding to high affinity environemntal PAH-binding site. These results challenge the significance of extrapopulations of desorption/nad bioavailability results that were obtained with PAH spiked soils. Further, a much higher PAH retention in historically contaminated soils suggests limited mobility and exposure of native PAHs. However, the addition/no co-solutes can reduce this retention and as a consequence, lead to a re-mobilisation of PAHs.

MO413 Ecological risk assessment of PAH contaminated area integrating bioavailability into Triad approach


One of the main threats to soil habitat and retention function is soil contamination with chemical pollutants, including PAHs. The high level of these pollutants may
cause an adverse effect on soil organisms and decrease soil biodiversity and quality. For assessing the risk from chemical contamination the ecological risk assessment (ERA) procedures are recently applied. The first stage of ERA is often based on the comparison of soil concentrations with soil screening levels (SSL) based on the total PAHs content determinations, which may lead to overestimation of the risk. The evaluation of the fraction of these compounds available to soil organisms gives a more realistic risk assessment procedure particularly in historically contaminated soils. The aim of the studies was to compare the results of ERA procedure based on three approaches: generic approach (comparison of total soil concentrations of PAHs with SSL), Triad approach based only on total PAHs and solid phase toxicity tests and Triad approach including toxicity of PAHs in soil water phase (relevant to the bioavailability of PAHs). The research area was located in the Niger Delta (100 km south of Port Harcourt, over 100 years) PAHs anthropopressure. The total concentrations of 16 PAH compounds were determined and compared to the soil screening values (the total content of 9PAHs) according to Polish soil guidelines. Risk assessment procedure based on Triad approach included: chemical, ecotoxicological and ecological measurements. The battery of tests was applied for ecotoxicological and ecological evaluations. The results were analysed and integrated in different lines of evidence, which allowed to calculate of integrated risk indexes. ERA assessment according to the generic approach indicated that almost half of research area is under ecological risk. Application in ERA procedure of the solid toxicity tests describing habitat function led to the selection of the limited area of high risk. However, incorporation to risk procedure toxicity test relevant to PAHs bioavailability permitted on more realistic assessment and action. Cyclodextrin was used as risk binding agent, ethanol as ink solvent and cement as core plant. The final assessment of ecological risk, after introduction of the tests describing bioavailability in risk assessment procedure, indicates on significant decrease of the area with high risk.

MO414 Bioaccessibility Extraction of Hydrophobic Pollutants: Benefits of Separating Leaching Agent and Acceptor Medium
D.J. Cocovi-Solberg; M. Miró, Universitat de les Illes Balears; A.P. Loibner, Boku, IFA-Tullin / Department for Agrobiotechnology IFA-Tullin; P. Mayer, Technical University of Denmark / Department of Environmental Engineering
Bioaccessibility extractions of organic pollutants from environmental solid samples are increasingly used in environmental risk assessment and management. Recent research has indicated that many bioaccessibility extraction methods have limited sink capacity for hydrophobic organic chemicals, which can lead to underestimation of bioaccessibility. Therefore, several studies have proposed to add a sink to the extraction medium, including the so called contaminant trap, the stagnant bed based sorptive bioaccessibility extraction and tenax beads-assisted extractions. While these methods certainly are a step forward, they also lead to challenges related to the separation of sink and matrix and/or the subsequent quantification of the bioaccessible fraction. The present study aimed at developing a new approach for (1) enhancing the sink capacity of bioaccessibility extractions, (2) improving phase separation and (3) facilitating the measurement of the bioaccessible fraction. Cyclodextrin was used as risk binding agent, ethanol as ink solvent and cement as core plant. Various physical formats of this configuration were developed and tested, and the simplest and highly performing format was further optimized and validated. This new configuration was characterized in terms of mass transfer kinetics, analytical performance criteria and suitability for direct analysis by high performance liquid chromatography (HPLC) and gas chromatography (GC). Finally, the developed method was applied to PAH contaminated soils and the results compared to results obtained with other existing methods.

MO415 PAH Composition of Clay Pigeon Target Fragments at Two Historical Skeet Range Sites
B.H. Magee, ARCADIS; G.C. Hoeger, ARCADIS / IEST; N. Forsberg, ARCADIS US Inc / IEST
In vivo bioavailability studies have been performed to determine the relative bioavailability of PAHs in clay pigeon target fragments at two historical skeet range sites. Target fragments, which are mixed with native soil, have elevated concentrations of PAHs that are expected to have reduced bioavailability compared to that seen in animal studies using pure benzo[a]pyrene in solvents added to rodent chow. The literature indicates that the clay targets are made from limestone with a binding agent such as petroleum pitch or coal tar, which serve as the source of PAHs. The literature, however, is unclear on the exact PAH mixture used in multiple site samples and the interactions with key soil components and environmental factors. Various sources have listed coal tar, coal tar pitch, bitumen, or petroleum pitch as the binding agent that may have been used in target production (ATSDR, 2002; EPA, 2003; ITRC, 2005). As part of the two bioavailability studies, PAH analyses were performed to characterize the PAH content of the fine fractions (~250 microns) of soil sites containing target fragments. PAH composition data from multiple site samples will be presented and compared to the PAH composition data of coal tar, coal tar pitch, bitumen, and petroleum pitch to provide insight on the source material for the PAHs found in soil sites at these range sites. The link of binding agents to specific target fragment samples will be used to evaluate data from the two individual bioavailability studies.

MO416 Assessing mobility potential of pharmaceuticals in bank filtration, surface and ground waters.
P. Adrian, A. BROUSSE, B. Journel, E. Beltran, CEHTRA SARL; F. Sahigara, P. Thomas, CEHTRA SAS
Bioaccumulation and soil adsorption studies for pharmaceuticals are required to determine if terrestrial (including ground water) risk assessment is required. Soil adsorption studies are carried out using batch equilibrium experiments according to the OECD 106 guideline. Additionally, the results may be used to refine the partitioning coefficients in the soil compartments and in sludge, the latter being the most important as it directly impacts partitioning in the sewage treatment plants (aqueous phase and sewage sludge). The combination between different intrinsic properties of pharmaceuticals, including ionisation potential may be useful to predict their occurrence in bank filtration, surface and ground waters. The aim of this poster is to present a simple model based on biological properties and physicochemical characteristics to predict the mobility potential of pharmaceuticals in the environment. The relevance of this model will be discussed using comparison of results with monitoring data from several countries.

MO417 Can the Complete Tenax Model be used to assess bioaccumulation at different trophic levels?
M.C. Archer, Southern Illinois University Carbondale / Center for Fisheries Aquaculture and Aquatic Sciences; A.D. Harwood, Southern Illinois University / Department of Natural Sciences; K. Huff Hartz, M.J. Lydy, Southern Illinois University-Carbondale / Center for Fisheries Aquaculture and Aquatic Sciences A major difficulty with characterizing sediment-associated hydrophobic organic contaminants is assessing the amount of compound that is available for chemical exchange with an organism. Tenax, a p-2,6-diphenylphenylenoxide polymer, has been successfully used as an extraction tool for estimating the bioavailability of hydrophobic organic contaminants in sediment, and has been applied to laboratory-spiked sediments, field-collected sediments, laboratory-exposed organisms, field-collected organisms and studies among laboratories. A literature-based model has been developed that describes the bioaccumulation of polychlorinated biphenyls (PCBs) from independently measured field-collected sediments. The primary objective of the current study was to evaluate the distribution and bioavailability of PCBs in a southern Illinois lake and to determine if Tenax extractable concentrations could be used to estimate bioaccumulation at different trophic levels, including higher level aquatic and terrestrial species. Species tested included tetragonathid spiders, emerging insects, oligochaete worms, midges, bluegill (male and female), largemouth bass, and channel catfish. This data revealed that PCBs in the lake's sediment were bioavailable to organisms inhabiting the lake, and that there was a relationship between animal tissue concentrations and Tenax extractable concentrations, which extends the potential use of the Tenax extraction method.

MO418 A look into the social acceptance of biochar-based soil remediation
A.A. Arenyeka, Newcastle University / Environmental Engineering
Contaminated soils and spills are currently major cause of concern in Nigeria. Remediation of organic pollutants by sorbent amendment has received increased research attention particularly over the last two decades. AC amendment has been tested extensively in the laboratory and the addition of a few percent AC by soil or sediment mass has been effective in sequestering PAHs, PCBs and Organochlorine pesticides (Ghosh et al., 2011). The study presented here is part of a broader interdisciplinary study which aims to investigate the viability of activated biochar as a sustainable technology solution for the remediation of oil pollution in Nigeria from a scientific as well as social point of view. The broader study involves comparing activated biochar with more traditional bioremediation technologies (biostimulation and natural attenuation) as well as subsequent risk assessment using the OECD 106 Contaminated Land Exposure Assessment (ELA) method and learning from Nigeria’s risk management framework. This paper will however focus on the social aspect of the research. This commenced with an extensive desktop study which provided an understanding of the physical, social and political context of crude oil spill remediation techniques in Nigeria and helped to identify principal actors and the rules which govern their decisions. Stakeholder interactions were carried out in face to face semi-structured interviews. Information obtained was analysed using thematic analysis and grounded theory. Major themes observed from the interviews include remediation challenges, technical considerations, pollution typology, environmental concerns, risk considerations & regulatory concerns. One interesting point is the likely potential of biochar as a viable choice for remediation of mangrove swamp due to the sensitive nature of the Niger-Delta ecosystem. Critical analysis of results obtained will be done using an adaptation of the IAD (Institutional Analysis and Development) framework (Ostrom, 1999). Finally, results from this study will be used in the final risk analysis in conjunction with results from the laboratory. Results would hopefully provide useful information for
dissemination to stakeholders particularly regulatory decision makers and would hopefully inform changes in the manner in which risk is currently assessed in Nigeria particularly as it relates to pollutant bioavailability.

MO419 Critical bioconcentration factor for short-term aquatic toxicity tests M. Kwon, U. University of Bonn; University of Excellence and Ecological Engineering, S. Lee, H. Kang, Korea University; B.S. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology

According to current regulations of chemicals management world-wide, massive amount of acute toxicity data are required for evaluating hazards and risks of chemicals. Current data requirements on the registration of chemicals are primarily based on the water solubility and their physical properties. For example, acute ecotoxicity data are required for chemicals with production volume greater than 10 ton/yr according to REACH. For highly hydrophobic chemicals, however, their internal concentration within the experimental duration might not be sufficiently high to cause acute effects because baseline toxicity is apparent when xenobiotics accumulate in the body greater than critical body burden. In this work, theoretical modelling of internal concentration vs. external concentration was conducted for standard acute ecotoxicity tests at three trophic levels – algae, invertebrate, and fish. It is unlikely that acute toxicity is observed for chemicals with bioconcentration factor (BCF) or octanol-water partition coefficient (Kow) as a surroage for biopartitioning greater than 107 in acute tests conducted following standard protocols. In order to evaluate the validity of the theoretical analysis, OECD's current EC regulation 2017/2018 (TGDs) agreed at 746/REACH, each manufacturer or importer of a substance in quantity of minimum one ton by year should submit a registration application to the European Chemicals Agency (ECHA). This indicates that current regulations about data requirements need to be revised for highly hydrophobic chemicals.

MO420 Relationship between IL structures and their (eco-)toxicological properties in view of ionic liquid registration in REACH. A. Bado-Nilles, UMRI SEBIO; G. Marliair, INERIS; C. Len, UTC ESCOM; P. Pandard, INERIS; J. Porcher, INERIS / UMRI SEBIO; S. ANDRES, INERIS

Ionic liquids (ILs), often termed as “green” solvents due to their extreme tunability of sought functional properties, are promoted by many stakeholders as green alternatives to the conventional volatile organic solvents. Nevertheless, this qualification was stated without sufficient caution versus versatility of ILs chemical structures which could induce significant variations in hazardous properties. Moreover, this class of compounds which contained organic cations and most inorganic anions may often present a high water solubility and poor biodegradability. These specific properties could influence the environmental behavior of these ILs and implied IL potential environmental and health effects. With regards to the exponential increase of applications, publications and patent for ten years, concerning ILs without halogen atoms, we may anticipate that some ILs could attain multi-ton industrial production in the near future. In this context, according to the EC regulation 2017/2018 (TGDs) agreed at 746/REACH, each manufacturer or importer of a substance in quantity of minimum one ton by year should submit a registration application to the European Chemicals Agency (ECHA). In this context, a global view of some physico-chemical, toxicological and ecotoxicological data available in the literature, in accordance with the information requested by REACH regulation, is proposed here. In fact, it seems to be important to identify the relationship between IL structures and their (eco-)toxicological properties to help manufacturer for design of the more safe IL structures.

Ecological Risk Assessment and Sustainable Management of Contaminated Sediment: Perspectives and Experiences (P)

MO421 Effects of the heavy metal zinc on nematode species: Validation of the NemaSPEAR[%]-index with acute multi-species and microcosm experiments A. Högénbäumer, Bielefeld University / Animal Ecology, S. Hös, Ecosas; K. Ristau, University of Bielefeld; E. Clas, Federal Institute of Hydrology BfG; C. Möhlenkamp, P. Heininger, Federal Institute of Hydrology, W. Traunspurger, University of Bielefeld

The nematode-based NemaSPEAR[%]-index was recently developed as a tool for assessing chemical induced changes of invertebrate communities in fine, adhesive freshwater sediments, that are known as hotspots for chemical exposure. In fine sediments, meiobenthic taxa (e. g. nematodes) are more abundant and species-rich than in macrozoobenthic communities and they are commonly used for assessing the biological quality of sediments. Thus, biomonitoring of this habitat should be increasingly based on the analysis of meiofauna to detect chemical induced effects. Based on the occurrence of nematode species in a pollution gradient, classifications in “species at risk” (NemaSPEAR) and “species not at risk” (NemaSPEARnotAR) could be made. In order to validate the NemaSPEAR[%]-index, experiments were carried out to assess the response of nematode species assemblages to a model chemical, the heavy metal zinc (Zn). Acute multi-species toxicity tests were performed to observe direct toxicity of Zn (20 and 50 mg Zn/l) on relevant nematode species, isolated from reference freshwater sediments. Moreover, long-term microcosm experiments were set up to study the effects of Zn (10 and 100 mg/kg dry weight) on nematode communities under more realistic conditions, including direct Zn toxicity and indirect food-web effects. Both results were compared to the species classifications in the NemaSPEAR[%]-index. The acute tests were only partly in line with the classifications of nematodes: in acute multi-species tests, individuals of Monhysteria paludicola (NemaSPEARnotAR) and Achromadora terricola (NemaSPEAR) showed highest sensitivity, while nematodes of the species Ironus ignatus (NemaSPEAR) and Tripyla setifera (NemaSPEARnotAR) were tolerant towards Zn. However, over the course of the microcosm experiment, dose-dependent changes in the nematode species composition along with a significant decrease of the NemaSPEAR[%] could be detected. The results showed that the NemaSPEAR[%]-index is clearly affected by chemical stress. Effects on the nematode community structure in the microcosm and in the field, however, might not only be triggered by direct toxicity, but also by indirect effects due to interactions within the complex environment of sediments. The present study supports the suitability of microcosms with natural meiofauna communities as a useful tool to assess the impacts of pollutants in freshwater sediments and open exciting possibilities for the future use of nematodes in the ecotoxicology.

MO422 Biodynamic modeling for contaminated sediments – a readily transferable tool to regulation? M. Casado-Martinez, Centre Ecotox; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology, B. Ferrari, University of Bielefeld

Sediments have several characteristics that make them a demanding environmental compartment to study. The large heterogeneity in its nature and composition encompasses a large variability in bioavailability and toxicity, challenging the development of environmental quality standards and effect concentrations for effective risk assessment. A proposed alternative is to use dose metrics as a measure of internal exposure such as bioaccumulation because, for a contaminant that cause toxicity, it is assumed that it must be first taken up and accumulated by the organism. For most organisms living in close contact with the sediment overlying water, pore water, sediment contact and diet all may contribute to some extent to the total load of a chemical substance in the body of the organism. Thus effective risk assessment for this environmental compartment needs to ideally use, however, that are capable of aggregating exposure through all relevant routes, taking into consideration species-specific biological features and natural history, and site-specific physico-chemical characteristics. [1, 2]. Biodynamic processes have been translated into conceptual and quantitative models that are capable to explaining differences in bioaccumulation patterns among species and environments for several metals. [3]. It is possible to derive bioaccumulation predictions as well as estimations of the relative prevalence of exposure routes by combining the quantified constants describing physiological processes with site-specific concentrations and geochemical conditions [3]. While biodynamics has become the new paradigm for describing bioaccumulation among scientists, it is recognised among the regulatory community as a promising powerful tool for improving ecological risk assessment. [2]. In the particular case of the sediment compartment, the use of biodynamic models is especially complex due to the differences in feeding and digestion strategies among benthic organisms and also due to the difficulty to determine ingestion rates and feeding preferences. The advances achieved over the last years will be reviewed to discuss on whether this level of development is enough for the implementation of this concept as a practicable tool for assessing chemical contamination and for the potential ecotoxicological and regulatory implications. [1] ECHA 2014. ECHA-14-R.13-EN. Helsinki, FI. [2] US EPA 2007. EPA 120/12-07001. Washington, US. [3] Luoma SN, Rainbow PS. 2008. Cambridge University Press, New York.

MO423 A protocol for assessing sediment toxicity in reservoirs before flushing L. Marziali, IRSA-CNR (Brugherio); L. Guzzella, Italian National Research Council Water Research Institute CNRIRSA; G. Tartari, IRSA-CNR Brugherio / UOS Brugherio; C. Bravi, S. Castelli, A.M. Ribaudo, Lombardy Region; C. Romani, R. Serra, Lombardy Regional Environmental Agency

Reservoirs are often characterized by high siltation rates which impair the storage capacity. Accumulated sediments are frequently removed by flushing, causing physical-mechanical impacts on aquatic organisms in the downstream rivers. In some cases chemical compounds (organic and metals) adsorbed on sediments may induce toxic effects, mainly in a long-term perspective. PrTox is a protocol for assessing toxicity of sediments for assessing the potential ecological and toxicological impact of reservoirs before flushing. It includes methods for sampling, chemical analysis and ecotoxicological evaluation of sediments, as well as criteria for risk assessment, based on cross-interpretation of results deriving from chemical and ecotoxicological analyses. In particular, different dilution rates of flushing silt are calculated in order to prevent adverse effects. The trial of the PrTox protocol application in some Italian reservoirs will be presented. Preliminary results show that the definition of threshold concentration may be biased by high natural background values (particularly for trace metals), by different on-site bioavailability and by synergistic effects of pollutants. Therefore, toxicity bioassays are essential to define the toxic potential of sediments. The protocol is an example of analytical approach useful for supporting decision
MO424 Application of complementary methods in chemical and ecotoxicological monitoring of sediments under WFD in Wallonia (Belgium).

Sediment remains important matrix for the monitoring of certain substances with significant potential for accumulation to assess long-term impacts of anthropogenic activity and trends. The EQS Directive (2008/105 EC amended by 2013/39/EU) provides that Member States should take measures with the aim of ensuring that exposures of contaminant in sediment will not significantly increase. Analyses of sediment can be a cost-effective approach for initial screening of areas for contamination. In using sediment as a first layer screening for certain chemicals, the monitoring programme may be downscaled. Indeed, the initial screening may help to identify areas of concern and areas where additional effort is needed.

Chemical analysis of priority pollutants is often not sufficient to explain ecotoxicological effects of these complex environmental samples. Risk assessment based on concentrations, e.g. of priority pollutants in sediments or water, obviously does not reflect the risk of the actual mixture of contaminants, but only the risk of those pre-selected toxicants. Bioassays are therefore useful tools for the evaluation of sediment in which both known and unknown contaminants are present. Effect-based monitoring is also useful for the development of investigative monitoring. Combining the three assessment methods (chemical, bioassay, ecology) in a Triad approach can give an answer that cannot be given by any of the individual methods by themselves. From 2010 to 2012, 31 monitoring stations included in the surveillance monitoring programme of the WFD in Wallonia (Belgium) were sampled for sediment. Bioassays (Chironomus riparius, Heterocypris incongruens for whole samples and Vibrio fischeri, Paracorvina artedi, Phylloicus cebus and Brachionus calyciflorus for pore waters) were carried out to determine the potential impact of contaminated sediment. According to their level of toxicity for the different tests, the sediments of the monitoring stations were classified from non-toxic to extremely toxic. Priority substances selected as pertinent for a monitoring in sediments and other pertinent chemicals were analysed in each sample (63 µm fraction). The link between physico-chemical and ecotoxicological parameters was assessed by multivariate statistical analysis.

MO425 Ecological risk assessment in a riverine subtropical area (Ribeira de Iguape River, SW Brazil) due to contamination from former Pb mining activities.
D. Abessa, Unesp / Marine Biology and Coastal Management; L.C. Morais, Universidade Estadual Paulista UNESP, F. Perina, Universidade Federal do Rio Grande / Institute of Oceanography; M.B. Davanzo, Ministry of Environment; L.M. Burnaem, São Paulo State University - UNESP Campus Experimental do Litoral Paulista; P.K. Guus-Choueri, UFRP, H.F. Puscidesa, UNISANTA; A.C. Cruz, São Paulo State University - UNESP Campus Experimental do Litoral Paulista; C. Araujo, Universidade de Aveiro / Biologist; V.G. Rodrigues, EESC USP; J.B. Sigolo, USP.

The Ribeira do Iguape River (RIR) is situated between the southwest of São Paulo and northeast of Paraná states (S-SW Brazil), and formerly represented an area of lead (Pb) extraction, comprising nine mines. The river received the direct discharges of mining residues, but in the early 1990’s the mining was ceased and the residues were deposited on the river banks, from where metals are still being released to the RIR. This investigation aimed to evaluate the ecological risks to the aquatic biota due to the release of metals from former mining activities into RIR. To achieve that, we used geochemistry and biological approaches. From 2009 to 2012, samples of the waters, sediments, suspended solids, bivalves (Corbicula fluminea and Anodontias tenebrosus) and mining residues were collected at six sampling sites along the RIR (P1 as reference site; P2-P6 influenced by the residues of mining). Waters and sediments were analyzed to the concentration of metals and toxicity to aquatic invertebrates. Bivalves were evaluated by bioaccumulation and biomarkers (lyosomal membrane stability and micrornucleus rate). Residues and suspended solids were analysed for chemistry, in order to indicate ecotoxicological risks in RIR due to metals. Mining residues exhibited high concentrations of metals, especially Pb, Zn, Cr and Ba, thus the residues were characterized as primary sources of contaminants. Concentrations of metals were often low in the waters, excepting to Fe, Al and Mn, which are naturally high. Sediments collected downstream to the deposits of residues were sandy but showed effects on the cell membranes and DNA, in animals from sites under influence the toxic levels. The suspended solids presented high levels of Pb, Ni, Cr, Cu, Zn. Soft tissues of both bivalves presented bioaccumulation by Pb, Cr and Cu, evidencing the bioavailability of metals and characterizing the exposure. Waters and sediments did not induce acute toxicity to Daphnia similis, but many sediment samples caused chronic effects. The analyses of biomarkers in C. fluminea showed significant effects on the cell membranes and DNA, in animals from sites under influence of mining residues. These effects correlated positively to the concentrations of metals in sediments. The results indicate that the RIR is passing through a process of natural recovery, but there are still ecological risks due to the suspended solids and sediments, as bioaccumulation and chronic effects are still present.

MO426 Methodologies and approaches for sediment characterization and ecological risk assessment based on experience gained in an Italian case study
L. Marziali, IRSA-CNR (Brugherio); L. Guzzella, Italian National Research Council Water Research Institute CNRISWA; m. carare; F. Scami, Italian Institute of Health; D. Rabuffetti, Regional Agency for the Protection of the Environment of Piedmont / Department of Verdiano Cusio Osola; M. Spanò, ARPA VCO; E. Bizzotto; S. Ceccon, ENSR Italia AECOM

The presentation will discuss the methodological experiences gained from an Italian case study undertaken under a risk-based approach, with details on adopted methodologies for the Environmental Risk Assessment and metric selection. The case study regards hysterosalpingoscopy. He discharge on a river that flows in a subalpine lake in Northern Italy and involves several investigations performed in collaboration with environmental agencies. Investigation design and data interpretation were based on a tiered approach to verifying at first step if it was necessary further investigations to evaluate the problem. Ecological risk assessment was the decision-making tool proposed in order to support the environmental management. Methodologies and criteria adopted in this case study are described as basis of further development of a general framework to deal such kind of comprehensive problem. The approaches illustrated below were shared in a technical board established to develop a guidance and address methodologies for Environmental Risk Assessment.

MO427 Eurocargo Venezia Ro-Ro Cargo Ship incident: evaluation of environmental adverse effect of wasted catalyzer with bioassays and bioaccumulation test
S. Macchia, ISPRRA Institute for Environmental Protection and Research; D. Sartori; S. Giuliani, ISPRA; L. Morroni, A. Gaion, ISPRÃ Institute for Environmental Protection and Research; Elutriates obtained from catalyst with bioassay were exposed to the unicellular alga Phaeodactylum tricornutum and the sea urchin Paracentrotus lividus, furthermore bioaccumulation test with polychaete Hediste diversicolor were performed. From Elutriates obtained from the catalyst showed high toxicity, greatly inhibiting the growth of algae (EC50 = 81.4) and causing a high percentage of abnormal plutei in P. lividus (EC50 ranging from 0.71 to 3.79). Only at a dilution of 1:100 any toxic effect was registered. A similar trend was also observed for water contained in the drums. Bioaccumulation tests with H. diversicolor showed that metals contained in the catalyst and released in the sediment may concentrate in the tissues of marine organisms and therefore potentially be transferred in the food web. The toxicity and bioavailability observed suggest the need to continue the monitoring activities in the abiotic and biotic environment in the medium term, in areas where the concentration of drums is still likely to be considered.

MO428 Sustainable dredged material management and support by multi-criteria decision analysis (MCDA).
W. Alihi, TU Hamburg-Harburg / Environmental Technology Energy Economics; A. Scheffler, Hamburg University of Technology

The port of Lübeck is one of Germany’s most important harbours for goods traffic to and from the Baltic Sea Region. Regularly maintenance dredging as well as capital dredging operations induced a chain of actions including taking the material out of the water, transport on land, and until its disposal. A range of solutions for sustainable dredged material handling exist and an assessment of these options often proves to be challenging for decision makers. MCDA provides decision support by processing different data sets and evaluating suitable options in a rational way. Using the stochastic multi-criteria acceptability analysis (SMAC) method with a modification of a previously developed computerised decision support in dredged material management, this study was performed to test decision support under uncertainty. The analysis endorsed that relocation to a dredged discharge pool and capping of an ammunition disposal site are viable options for Lübeck port. On the other hand, the use of dredged material from port expansion and its disposal on land are considered to be of very low sustainability under the given circumstances. The case study demonstrates the capabilities as well as boundaries of computer-aided decision making under the premise of incomplete information. Despite uncertainties, sustainable decision making is possible with appropriate MCDA methodologies although minimum requirements concerning both data quality and data quantitum must be fulfilled. Consequently, one possible alternative could not be integrated into the case study.
This study aimed to use the Triad approach (SQT) to define the best disposal site of dredged material of a maritime terminal in Brazil. The study area consisted of a polygon measuring 12km², located approximately 3 km from the Terminal on the northeastern coast. This area comprised of 22 stations sampled in February 2013. At each station sediment samples were collected for chemical analysis (arsenic, cadmium, lead, mercury, nickel, zinc, chromium, iron, copper, PCBs, endrin, diendrin, DDD, DDE, DDT, HCH, chlordane, methyl naphthalene, benzo(a)anthracene, anthracene, benz(a)pyrene, chrysene, dibenz(a,h)anthracene, phenanthrene, naphthalene, acenaphthene, fluorethane, fluorene, pyrene, PAHs and BTB) for ecotoxicological analysis (percentage of impairment of reproductive Nitokra sp in the samples compared to control) and for benthic community structure analysis (total number of organisms, diversity, evenness and species richness). For the Triad Approach the very non-target organisms integration method - RTM proposed by Del Valls and Chapman (1998), the Ranking Scale method proposed by Carr et al. (2000) and the Decision Table method proposed by Chapman (1990) were used. Based on the SQT evaluation methods it was observed that sampling stations 5 and 15 presented more alterations than the other stations. The sampling station number 5 presented high chemical contamination, particularly cadmium and PAHs. It was also observed that benthic organisms found in station 15 are more affected than station 5. For the sampling station 15 the found rating can be explained mainly by the ecotoxicity and the high cadmium levels. From the Decision Table it was possible to conclude that no sampling stations presented alterations in the benthic fauna assemblages. 

Impact of statistical methods used in ecotoxicological studies on regulatory decisions (P)

MO432 Challenges for Statistical Analysis of Ecotoxicology Experiments J.W. Green, DuPont / Applied Statistics Group; T.A. Springer, Wildlife International, Ltd.; J.P. Staveley, Exponent The conceptual advantages for ECx estimates from regression models fit to concentration-response data are well discussed and toxicity assessment criteria are clear. There is little disagreement that fitting a regression model to data and reporting a derived ECx value is advantageous for “typical” concentration-response tests. However, the amount and importance of ecotoxicity testing that is not “typical” concentration-response testing is widely unappreciated. Limitations on determining, interpreting, and applying ECx values become obvious to practitioners who are routinely confronted with “quirky” concentration-response testing situations. We present evidence that the criticisms of NOECs and the advantages of ECx estimates have both been exaggerated. While ECx estimates are certainly valuable, even preferable, under the right conditions, those conditions do not always exist and it is important to recognize such situations and bring to bear the right tools for a wide range of responses from many types of ecotoxicity experiments. There are often extreme limitations in the number of concentrations, reps per concentration, and number of subjects per rep that can be tested. Very expensive equipment of limited capacity is needed for many modern experiments and this restricts many experiments to 4-5 test concentrations plus control with 2 or sometimes 4 reps/concentration. This is a problem, since generally a larger number of concentrations is needed for regression and ECx determination than for NOEC determination. While in the abstract, better curves are obtained by extending the range of test concentrations, limitations arise from the low solubility common to many test chemicals, since test concentrations are restricted by the limit of solubility and, at the low end of the range, the limit of quantitation (LOQ). Further limits are imposed by the difficulty in maintaining stable test concentrations and analytical variability. Even some normality “well behaved” endpoints cannot always be fit by a model that gives meaningful results. Without NOEC, there may be no means to assess the data from such studies. Appropriate statistical models and tests demonstration that hypothesis testing offers more than is often perceived. Responses for which no regression models have so far been proposed or accepted within the ecotoxicology community also demand a NOEC.

MO433 From NOEC to ECx: a large scale data analysis on ecotoxicological studies with pesticides F. Marchetto, F. Galiberti, ICPS; W. de Boer, H. van der Voet, Alterra Wageningen University and Research Centre; A. Moretto, State University of Milano, INSTM; S. Fabbri, University of Milano 

Historically chronic or long-term studies performed for the authorisation of plant protection products (PP) result in the reporting of endpoint values in terms of No-Observed Effect Concentration (NOEC). The related Test Guidelines (OECD, ISO, EPA) were designed to fulfil the data requirement for NOEC determination. NOEC values are however criticised since their values strongly depends on the experimental study design and actual extrapolation of Effect Concentration affecting a percentage x of the test organisms or where a percentage x of an effects is observed (ECx) is considered more appropriate since it takes into account the whole concentration-response curve. The new Regulation (EC) No. 1107/2009 for
the authorisation of PPPs and the related data requirements (Commission Regulation (EU) No. 283/2013 and 284/2013) require that ecotoxicological endpoint data from chronic or long-term studies provided by the Applicant are reported as ECx or ECx<sub>20</sub> values together with the NOEC. However, there is no systematic comparison available to compare NOEC values to EC<sub>20</sub> and EC<sub>50</sub> values derived from the same study. In the present work, 200 long term and chronic studies on aquatic organisms, equally distributed among fish, daphnids, algae, aquatic macrophytes and invertebrates, were re-analysed in order to calculate NOEC, EC<sub>20</sub>, EC<sub>50</sub> and their limit of confidence (from the same study) using appropriate statistical analyses. A comparison of NOEC with EC<sub>50</sub> values and their lower limit of confidence is performed by analysing the distribution of the NOE/EC ratios. Considerations are made on studies based on the same organisms and on the study design (appropriately developed to calculate EC<sub>20</sub> for algae and macrophytes and NOEC for fish and daphnids).

MO434

Does the choice of NOEC or EC10 affect consequences of ecological risk assessments?

Y. Ikeda,
Research Center for Life and Environmental Sciences; K. Kotani,
Yokohama national university / Graduate School of Environment and Information Sciences; S. Kashiwada, Toyo University / Department of Life Sciences; S. Masunaga, Yokohama national university / Faculty of Environ Information Sci

No observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) have been used in ecological risk assessments but strongly criticized for being linked to some percent effect in the data of the endpoint. This means that high control variability in an experiment may result in higher NOEC values. As a result, the NOEC or that this percent effect is biologically meaningful in general for all test organisms. The non-equivalence of statistical significance and practical significance does not allow to conclude the equivalence of statistical significance and practical significance and the inclusion or exclusion of specific data points into the analysis. Therefore, the question of whether NOEC or EC10 affects hazardous concentration for 5% of species (HC5) estimated using species sensitivity distribution approach.

MO435

ECX-Values in Ecotoxicology - Defining endpoint-specific and biologically meaningful effect concentration threshold values (ECx)

M. Caspar, BASF SE / Regulatory Ecotoxicology; E. Salinas, BASF SE; L. Peters, BASF SE

For more than 2 decades ecotoxicologists have debated replacing hypothesis testing endpoints (NOEC and LOEC) with regression-based values such as E(L)C(x). The main criticism of the NOE/EC approach is the complete dependence on the chosen test concentrations and no inherent correspondence to specified no-effect levels. Thus, the NOEC is widely (mis-) used as a zero-effect concentration, although it can be linked to some percent effect in the data of the endpoint. This means that high control variability in an experiment may result in higher NOEC values. As a result, the growing interest within OECD member countries and the scientific community, the regression-based ECx-approach was supplemented in several regulations, guidance documents and guidelines as an alternative for the NOEC-approach for ecotoxicity test systems. It does not depend on arbitrarily chosen test concentrations and is a concentration-effect relationship into account, including the calculation of confidence intervals. Until now, the REACH guidance document (R.10) suggests an EC10 as a substitute for the NOEC, whereas the regulation No. 283/2013 (in line with EC No. 1107/2009) for PPP’s recommends an EC10 or EC20 for the Daphnia magna reproduction test (OECD TG 211). But there is no scientific reason to presume that a 10-20% effect relates to the NOEC or that this percent effect is biologically meaningful in general for all endpoints in all test organisms. The non-equivalence of statistical significance and biological relevance has long been recognized and in the best interest of environmental protection it is indispensable to detect threshold values in relation to population-level significance. Therefore, the question of which magnitude of effect is important to detect is not a statistical but rather a biological issue. The 5th percentile for the distribution of chronic, no-effect aquatic toxicity data, referred to as the HC5, can be utilized as a concentration threshold that is designed to protect ecosystem function, populations and communities in the natural environment. There are many statistical methods that can be used to estimate the HC5 and the associated confidence intervals. These methods include non-linear curve fitting, linear regression, classical distribution fitting, Bayesian distribution fitting, and non-parametric bootstrapping. Given that SDs are routinely based on a limited number of species, inferences regarding the distribution of the data are largely based on professional judgment and assumptions. In addition, these calculations are often performed using tools that were not developed by the user, insulating the user from the underlying methods and assumptions and often providing a false sense of accuracy. When comparing a fitted SSD to the experimental data, the plotting position of the experimental data is sensitive to the method used to calculate percentiles, therefore affecting the assessment of the quality of the fit. In addition to these methodological concerns, quality review of the underlying data may be more or less rigorous and certain functional groups may be over- or under-represented. These factors can significantly impact the selection of the final dataset and the calculated HC5. To illustrate these issues, SSDs and HC5s were calculated for triclosan using a variety of methods and a variety of data handling practices. Sensitivity analyses were conducted to demonstrate the significance of methodological considerations as well as the inclusion or exclusion of specific data points in altering the estimate of the HC5.

MO436

Better by design - improving similarity in field microcosm studies

C. Jenkins, HLS / Ecotoxicology; E. Hopkins, HLS; E. Quinton, HLS / Aquatic Ecotoxicology and Biodegradation; P. Xirogiannopoulou, Huntingdon Life Sciences Ltd

Field microcosm study designs follow well established guidelines (HARAP, CLASSIC, OECD and EFSA) with relatively standard methods for sampling and enumerating primary producers, invertebrates and emerging insects. In the current climate where the effectiveness of these studies is driven into sharp focus by the need to highlight the power of statistical analysis to identify significant change (e.g. case-study MDD, EFSA 2014) it is clear that there is a need to re-examine study methodology. In particular, we need to examine the effectiveness of methods to establish and monitor invertebrate populations and to control the similarity of experimental systems in order to improve the sensitivity and reliability of the applied statistical methods. In our studies, invertebrates are collected from field sites or un-treated reservoirs and added to standardised microcosm units (30 cm deep) systems containing submerged and emergent macrophytes designed to simulate edge of field ditches. A number of methods have been introduced to achieve and subsequently control the similarity of macro-invertebrate populations during the establishment. Invertebrates such as Asellus and Crangonyx are collected by sweep-netting into a number of containers, the collection pooled, mixed and sub-samples randomly allocated to microcosms. For taxa such as Chaoborus and standardised collections of larvae are reared and added to microcosms directly (without sub-sampling) as are Cleoem dipterum nymphs. Using at least two quantitative counts of the numbers in invertebrate samples, numbers are adjusted to improve similarity either by additional collection (for Chaoborus larvae) or by the exchange of invertebrate samplers between microcosms. For emerging insects, we
Biodegradation and Environmental Fate of Chemicals - Regulatory Acceptance of Non-Standard Tests (P)

MO439
Study design and statistical analysis of regulatory community ecotoxicity studies
A. Lawrence, Cambridge Environmental Assessments; K. Brown, Independent Consultant; G. Trumpton, University of Southampton; P. van den Brink, Alterra Wageningen UR / Aquatic Ecology and Water Quality Management Group

Ecotoxicological field studies conducted under EC Regulation 1107/2009 may be designed by industry notifier in association with external consultants and contract research organisations (CROs). The results may then be interpreted and summarised before inclusion in a regulatory submission in support of a product or active substance registration. The submission will be reviewed by representatives from Member State (MS) Competent Authorities, in terms of both study quality and meaning. Not all parties involved may be familiar with the statistical design and interpretation requirements of such studies, which are complex. Here we discuss certain important aspects of the design and statistical analysis of such studies. These include impact of spatial scale on effects and recovery in terrestrial plots, analysis of study data by sampling method or functional group, investigation of abundance data alongside statistical analysis to aid interpretation, repeated sample testing, and use of Principal Response Curves including analysis of reference items, amongst others. This work was funded by UK Chemicals Regulation Directorate.

MO440
A critical evaluation of the weight of evidence approach for determining persistence in PBT assessments under REACH
R.J. Brown, wca consulting; M. Claessens, DuPont; I. Colombo, Solvay Specialty Polymers; M.R. Embry, ILSI Health & Environmental Sciences Institute (HEI); B. Hidding, BASF SE; S. Jacobi, Albenmarle Europe / HSE; M. Leun Paunen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences TES Division; N. McGrath, Euro Chlor; C. Miyata, Sumitomo Chemical Co Ltd; G.C. Roberts, ENVIRON UK Ltd / Brixham Environmental Laboratory; J.R. Snape, AstraZeneca UK Ltd / AstraZeneca Global Environment; K.B. Woodburn, Dow Corning Corporation / HES; V. Vrijhojf, ECETOC

This presentation gives a critical overview of the available tests for evaluating persistence using a weight of evidence approach under the revised Annex XIII of the EU REACH Regulation. The technical guidance for this updated regulation is currently being developed as a support document of the EETM project. A critical review of the EETM supporting documentation and supporting documents of national and international reports suggesting approaches for PBT assessment following the updated regulations which is used as the basis for this presentation. The limitations of the standard OECD 301 series ready biodegradation tests as screening tools for persistence will be outlined as well as potential enhancements that can be used to improve environmental realism and to evaluate if a substance is likely to require further testing. Suggestions on how inherent and marine biodegradation tests should be interpreted in the context of PBT assessment is given. Simulation studies may be required to refine the persistence assessment of a substance if the criteria for the ready and inherent biodegradation studies are not met. The relevant simulation study should be designed based on the relevant compartment of concern, the properties of the test substance and using realistic environmental temperatures. The OECD 308 test was developed as a tool for assessing spray drift for pesticide applications and is not a river simulation study and therefore is not necessarily relevant for all substances released into rivers, because the test system is static and the water to sediment ratio is very low. Alternative tests such as OECD 309 or OECD 314 may be more appropriate tests for ‘down the drain’ chemicals. In simulation studies the persistence of degradation products should also be considered. Some of the difficulties associated with generating and interpreting simulation studies for PBT assessment will be highlighted. Recommendations concerning non-extractable residues (NERs) are also provided. NERs are expected to be strongly bound to sediment or soil and, while adsorbed, they are protected from degradation and are not bioavailable. Therefore, in the context of the PBT assessment, NERs should be considered not persistent, bioaccumulative, or toxic. In conclusion, while there is a clear decision tree for conducting ‘P assessment supported by a number of validated tests, in practice the available studies are not necessarily fit for purpose and may result in chemicals being wrongly assigned as PBT or vPvB.

MO441
Ready biodegradability of poorly water soluble substances: Impact of inoculum and bioavailability improvement methods on ready biodegradability of two chemicals
C. Sweetlove, L'Oreal SA / Research and Innovation; J. CHENEBLE, L'Oreal; Y. BARTHIEL, EUROFINS / Expertises Environnementales; F. ESCAFFRE, L'Oreal Research / Research and Innovation; M. BOULAM, EUROFINS / Expertises Environnementales; G. THOUAND, University of Nantes / UMR CNRS GEPEA CBAC lab; J. L'HARIDON, L'Oreal Research / Research and Innovation

The biodegradation of chemicals in the bioremediation process of polluted groundwater and toxic waste sites requires a close physical contact between bacteria and the pollutant. Therefore, a low hydrophilicity may lead to underestimate the chemical’s biodegradability when tests are performed in water. For example, a lipid compound with a density below 1 floats at the air-water interface in the test vessel. In this configuration, the bioavailability of the chemical to the bacterial cultures can only be assessed by a few experimental set-ups, such as suggested technical adaptations to improve the bioavailability of poorly water-soluble chemicals. Their implementation could improve the biodegradation results. The aim of this study was to define the impact of different inocula on biodegradation of two chemicals (a powder and a liquid) in a ready biodegradation test (RBT). Three inocula were selected: two specific bacteria (Nitrosomonas europaea and Corynebacterium pseudophælum) and an activated sludge from a wastewater treatment plant. In addition, seven Bioavailability Improvement Methods (BIM) were compared with these three inocula. The BIM performed are dispersion with ultrasound (10 and 30 min), emulsion with 2,2,4,4,6,8,8-Heptamethylnonane (HMM), silicon oil addition (two concentrations), dispersion with silicon oil associated to ultrasonic treatment (two different operating conditions) and direct addition to the test flasks (Silicon Oil®). Furthermore, the degree of mineralization was determined by screening RBT tool (Respendis VI®), which allows to compare a high number of operating conditions in the same test with the same microbial inoculum with his 95 test flasks (volume: 150 mL). This poster highlights the best inoculum to compare BIM for test chemicals (one powder and one liquid) and the best BIM to improve the biodegradation of the two test chemicals.

MO442
Biodegradation of Triclosan during sewage treatment plant processes
S. Pawlowski, S. Champ, BASF SE

Triclosan is an antibacterial biocide which is commonly used in various cosmetic and personal care products, such as hand wash or tooth paste since decades. Due to these down-the-drain applications, the main entry part into the environment is via sewage treatment plants (STP) effluents. Thus, the removal efficiency of the STP is key to the STP effluent concentrations of Triclosan and as a consequence to the concentrations in the river. Triclosan is considered as not readily but inherently biodegradable based on available OECD 301 and 302 study results. Due to these test results, the discussion continues whether the removal of Triclosan during the STP process is mainly based on biodegradation or on adsorption. In order to provide more detailed information on the environmental fate profile of the substance within an STP, the results of two continuous activated sludge (CAS) studies under aerobic conditions are presented. The test concentration ranged from 40 to 2000 µg/L and 7.5 to 50 µg/L, respectively, for both studies. The overall removal efficiency ranged from 98.5 – 99.3 % for the 40 µg/L and 99.0 – 99.7 % for the 7.5 µg/L test series. The degree of mineralization to CO₂ ranged from 74 – 90 %. The amount of substance being associated with the biomass (including non-extractable residues) ranged from 1.5 – 17 %. Furthermore, results from a biodegradation screening test (OECD 301B) on Methyltriclosan demonstrate that this transformation product of Triclosan is inherently biodegradable. The results clearly demonstrate that Triclosan and its transformation product are extensively biodegraded during the activated sludge process within a sewage treatment plant.

MO443
Screening test systems for the determination of biodegradation kinetics in water, water-sediment and soil - development and applicability in the regulatory context
T. Junker, ECT Ekotoksikologische GmbH; A. Coors, ECT Ekotoksikologie GmbH

Biodegradation data of chemicals are needed across different regulatory frameworks, inter alia for the assessment of persistence, exposure and risk. Besides experimental data, the prediction of degradation rates, half-lives and pathways by models plays a decisive role. For these reasons, these models were mainly developed based on biodegradation screening test data (e.g. OECD 301) and not on biodegradation data (e.g. OECD 303) related to water-only test systems, whereas models based on quantitative biodegradation data for more complex environmental matrices such as soil and sediment are lacking. To overcome these shortcomings, two new screening test systems for the determination of the ready biodegradability in water-sediment systems (WSSST = Water-Sediment Screening Tool) and soils (SST = Soil Screening Tool) were developed based on OECD 301C (MITI-Test).

Biodegradation experiments were performed in water (MTI), water-sediment (WST) and soil (SST) for a set of 15 compounds. The degradation curves were determined by non-linear regression models and the kinetic parameters 'mineralization at the plateau', 'duration of the lag-phase', 'mineralization half-life' and 'degradation rate constant' were determined using the regression model with the best fit. The test systems MITL, WST and SST were evaluated for their suitability to provide quantitative biodegradation data and kinetics that can be applied in the regulatory context. The main focus was on the persistence assessment within REACH, where degradation half-lives from simulation studies shall be
directly compared to trigger values for water, sediment and soil to identify persistent compounds. However, for many substances there are no data from simulation studies available. Thus, a screening assessment for persistence is performed under REACH that is mainly based on ready biodegradability tests according to OECD 301 and extrapolation of these data to derive half-lives for water as well as soil and sediment by multiplication with default factors. In addition to experiment, a mineralization half-lives, alternative approaches described in literature and soil and water half-lives were used in the packages to determine half-lives for the 15 test compounds in water, sediment and soil. By means of these data, a screening assessment for persistence was performed by comparing the half-lives to the persistence criteria under REACH.

MO444 Sorption and degradation of pharmaceuticals in soil and biosolids amended soil M. Vilà, M. Hidalg, University of Girona / Department of Chemistry Pharmaceuticals are emerging contaminants which have drawn concerns about possible harmful effects in the environment or the potential entry of drugs into the human food chain. Most studies on the environmental fate of pharmaceuticals have focused on their behaviours during wastewater treatment processes, the sludge and in environmental waters. However, there is still a significant lack of knowledge of their transport and fate in agricultural soils. Sewage sludge from wastewater treatment plants (WWTPs) is commonly used as soil amendment because it contains nutrients and organic matter which can improve the soil quality. Since pharmaceuticals are routinely found in biosolids from WWTPs, once land applied they are potentially mobile and available for plant uptake and bioaccumulation. The fate of pharmaceuticals in soils is mostly determined by sorption and degradation processes. This group of contaminants consists of a wide range of compounds. Their sorption behaviours vary from compound to compound and are difficult to predict because is often controlled by interactions with specific functional groups and plant root exudates. Moreover, land applications of biosolids can alter the chemical and physical properties of the soil, with an increase in soil-water retention and organic matter content which can consequently affects the interaction with chemicals. In this study, laboratory experiments were performed to investigate the sorption and degradation of three anti-inflammatory (naproxen, ibuprofen and diclofenac) and five antidepressant drugs (citalopram, fluoxetine, norfluoxetine, paroxetine and sertraline), with different physicochemical characteristics, in an agricultural soil. The effect of biosiland amendment on sorption and degradation was also examined. As expected, antidepressants adsorbed very strongly to the soil with and without addition of biosolids. Adsorption tests using a batch equilibrium method showed that adsorption of all tested pharmaceuticals could be well described by a linear isotherm with a positive correlation. After mixing the soil with biosolids, a significant increase in the sorption capacity of the soil for all the compounds was observed when the pharmaceuticals coexisted. This coludative effect was no observed for anti-inflammatory drugs in absence of antidepressants. Biosolids amendment increased the sorptive strength for all the compound studied and, in general, had also an effect on degradation under the conditions of this study.

MO445 Bioremediation of Hydrocarbons with Microorganisms S. Ashraf, Environmental studies center-QU; M.Y. Al-Sulaiti, ExxonMobil Research Qatar / Water Reuse; C.S. Warren, ExxonMobil Research Qatar / Advanced Environmental Scientist; I.M. Al-Shaiikh, Environmental Studies Center-QU Bioremediation or the use of microorganisms for environmental cleanup is usually the preferred and major route of PAH removal from contaminated environments because it is cost effectiveness and complete cleanup (Pothuluri and Cerniglia, 1994). Several isolated microorganisms have been successfully utilized in major hazardous waste cleanup processes such as, in industrial processes streams and effluents (Levinson et al., 1994 and Al-Wasify and Hamed, 2014). Unfortunately, most of these studies were carried out in Western countries, to a limited extent in South America and Asia (Prantera et al., 2002). In Qatar, there is limited information on microbial degradation of polycyclic aromatic hydrocarbons (Al-Thani et al., 2009). In this work, we reported the isolation and characterization of some bacterial and yeast species from soils and industrial wastewater (IWW) collected from an oil refinery and a wastewater treatment plant (WTP) of El Chalten, Santa Cruz, Argentina. The fraction was obtained by sequential dissolution using hydrochloric acid, sodium hydroxide and hydrofluoric acid and purified by dialysis in cellulose tube Sigma D-7884. A solution of 50µg/ml of HA plus 0.04 mM Rf was used as sensitizer. Overall, it was observed that the visible-light-mediated photooxidation of 3-OHP at pH 7, sensitized by low Rf and HA concentrations, and standardized by comparison to the photooxidation rate of the known oxidizable target FFA, is an efficient process. It should be mention that the experiments of oxygen uptake for 3-OHP and FFA were made under identical experimental conditions, including the concentration of the oxidizable substrates. On the other hand, the degradation of POH only operates in the alkaline pH range. A moderate decrease in the rate of oxygen consumption by 3-OHP upon Rf +HA sensitization was observed as compared to the simple addition of the oxygen uptake rates for 3-OHP upon individual Rf and HA sensitization. It is attributed to a catalytic decomposition of O2− by HA, which competes with 3-OHP by the oxidative reaction (see 1: 0.25(1 – br clear=“all” />[1] J.I. Edges, J.M. Oades, Comparative organic geochemistry’s of soils and marine sediments, Org. Geochem. 27 (1997) 319–361. [2] M.C. Scapini, V.H. Conzonno, V.T. Balzarotti, A. Fernández Cirile, Comparsonof marine and river water humic substances in a Patagonian environment (Argentina), Aqaut. Sci. 72 (2010) 1–12. [3] Mac Carthy, P., R.L. Malcom, A proposal to establish a reference collection of humic materials for interlaboratory comparisons, Geoderma, 16, pp 179-181 (1976).

Engineering in vivo feel model systems for advancing environmental hazard and risk assessment (P)

MO446 Yeast Identification from bioMérieux was used to identify the most common yeast isolates which show thirteen species which were belonged to eight different genera. The most common were Candida spp., Trichosporon mucoides and Cryptococcus spp.

MO446 Effects of HP-β-CD concentrations and repeated exposures to diesel on biodegradation of benzo[a]pyrene in soil I.S. Obeuke, University of Benin Benin City Nigeria; O. Adebiyi, UNIVERSITY OF ILORIN NIGERIA; K.T. Semple, University of Lancaster / Lancaster Environment Centre Aim: It is well-known that low aqueous solubility and poor microbial catabolic activity limit benzo[a]pyrene (BaP) biodegradation in soil. Used as a solubility-enhancement agent, the effect of increasing hydroxypropyl-β-cyclodextrin (HP-β-CD) concentrations (0, 12.5, 25 and 50 mM) on benzo[a]pyrene mineralisation was assessed in soil. The effect of repeated exposures over 150 d to diesel oil (1x500, 1x5000, 2x250, 2x500, 5x100 and 1x5000 mg L⁻¹) on the development of benzo[a]pyrene catabolic activity was also assessed. Materials and Methods: Sequential aqueous, HP-β-CD and alkaline extractions were used to quantify benzo[a]pyrene bioavailability and partitioning in soil. Standard radiometric assays were used to measure catabolic activity and mineralisation of ¹⁴C-benzo[a]pyrene in soil. Results: Indigenous catabolic activity towards benzo[a]pyrene was low in control soil (mineralisation extent < 1%). However, pre-exposure to diesel oil significantly enhanced (11–55times) catabolic activity; this biodegradation was modelled as a first-order decay equation. Conclusions: Increasing HP-β-CD concentration significantly increased (26–67times) benzo[a]pyrene bioavailability in soil, it failed to enhance benzo[a]pyrene mineralisation; however, lower HP-β-CD concentrations appeared to have some impact on mineralisation. Overall, whilst the presence of 50 mM HP-β-CD significantly increased (26–67times) benzo[a]pyrene mineralisation in diesel-contaminated soil. These findings are important in assessing the fate of benzo[a]pyrene and designing bespoke remediation strategies for soils chronically exposed to petroleum-derived oils.

MO447 Humic acids of Patagonian soils. Isolation and kinetic study of the photochemical action in the natural degradation of environmental pollutants M.M. Bregliani, J. ESCALADA, UNIVERSIDAD NACIONAL DE LA PATAGONIA AUSTRAL; A. Pajares, UNPATAGONIA; N.A. GARCIA, UNIVERSIDAD NACIONAL RIO CUARITO Objective Humic substances, among which humic acids (HAs) constitute a relevant fraction, play an important role on the development of benzo[a]pyrene catabolic activity in soil. Conclusions Several models for low-OH diesel contaminants. phenol (PhOH) and 3-hydroxypropyline (3-OHP) were employed. The HA was isolated from a forest soil near El Chalten, Santa Cruz, Argentina. The fraction was obtained by sequential dissolution using hydrochloric acid, sodium hydroxide and hydrofluoric acid and purified by dialysis in cellulose tube Sigma D-7884. A solution of 50µg/ml of HA plus 0.04 mM Rf was used as sensitizer. Overall, it was observed that the visible-light-mediated photooxidation of 3-OHP at pH 7, sensitized by low Rf and HA concentrations, and standardized by comparison to the photooxidation rate of the known oxidizable target FFA, is an efficient process. It should be mention that the experiments of oxygen uptake for 3-OHP and FFA were made under identical experimental conditions, including the concentration of the oxidizable substrates. On the other hand, the degradation of POH only operates in the alkaline pH range. A moderate decrease in the rate of oxygen consumption by 3-OHP upon Rf +HA sensitization was observed as compared to the simple addition of the oxygen uptake rates for 3-OHP upon individual Rf and HA sensitization. It is attributed to a catalytic decomposition of O2− by HA, which competes with 3-OHP by the oxidative reaction (see 1: 0.25(1 – br clear=“all” />[1] J.I. Edges, J.M. Oades, Comparative organic geochemistry’s of soils and marine sediments, Org. Geochem. 27 (1997) 319–361. [2] M.C. Scapini, V.H. Conzonno, V.T. Balzarotti, A. Fernández Cirile, Comparsonof marine and river water humic substances in a Patagonian environment (Argentina), Aqaut. Sci. 72 (2010) 1–12. [3] Mac Carthy, P., R.L. Malcom, A proposal to establish a reference collection of humic materials for interlaboratory comparisons, Geoderma, 16, pp 179-181 (1976).
In most gonochoristic fish species, aromatase, the enzyme responsible for the synthesis of estrogens, has been shown to play a critical role in the process of sexual differentiation (SD). In zebrafish, that undergoes a juvenile intersex stage, there is still a lack of knowledge regarding the precise localization and pattern of expression of the aromatase gene (Cyp19a1a) impeding the formal characterization of the role of Cyp19a1a in this process. To fulfill this gap, the spatio-temporal expression pattern of cyp19a1a was analyzed in a novel transgenic zebrafish model expressing Green Fluorescent Protein (GFP) under the control of the zebrafish cyp19a1a gene promoter. First, we showed a perfect co-expression of GFP and endogenous Cyp19a1a protein in adult gonads that was localized in the cytoplasm of oogonia, oocytes and yolk sac cells of transgenic male and female larvae. Then, the spatio-temporal expression pattern of cyp19a1a was studied during SD using GFP fluorescence imaging in gonads at 20, 30, 35 and 40 day post-fertilization (dpf). Our data showed that GFP was expressed in all undifferentiated gonads of 20 dpf-old zebrafish. At later stages of development, GFP expression increased in early differentiated female at 30 and 35 dpf to reach high GFP intensity in well-differentiated ovaries at 40 dpf. This pattern of gonadal GFP expression remarkably differed from that of males which consistently displayed low GFP expression as compared to female whatever their stage of development, resulting in a clear dimorphic expression between males and females.

Interestingly, fish that undergoes ovary-to-testis transition (35 and 40 dpf) presented an intermediary GFP level as compared to those measured in males and females. Our results suggest that (1) cyp19a1a is expressed early during gonadal development before the histological process of SD, (2) the down-regulation of cyp19a1a expression is critical for the testicular differentiation, (3) although cyp19a1a expression exhibit a clear dimorphic expression in gonads during SD, its expression persists whatever the sex suggesting that estradiol synthesis is important for gonadal development in both male and female. Monitoring the expression of the GFP reporter and control an endocrine disrupting chemical will help to identify the interest of this model to study the mechanisms of action of endocrine disrupting chemicals on critical physiological processes such as SD.

MO445
Application of transgenic zebrafish to enable adverse outcome pathway analysis for endocrine disrupting mechanisms

A. Brown, Exeter University / Safety Health Environment; J. Green, University of Exeter; M.J. Hetheridge, University of Exeter / Biosciences; R. Currie, Syngenta; C.R. Tyler, Biosciences College of Life and Environmental Sciences

Around 800 chemicals are known, or suspected, to interact with hormone receptors, and to affect the development of male and female gonads. However, only a small fraction of these chemicals have been investigated in tests capable of identifying overt endocrine disrupting adverse initiating events (MIEs). However, only a small fraction of these chemicals have been investigated in tests capable of identifying overt endocrine disrupting adverse effects in intact organisms or their progeny or (sub)populations (Weybridge, 1996, WHO/UNEP, 2012). Whilst in silico and in vitro tools may provide some indication of the potential endocrine activity of chemicals, reliable in vivo systems are required to assess for potential adverse effects, ideally in a high throughput/high content manner. Transgenic animal models (e.g. zebrafish) offer systems whereby MIEs that trigger adverse outcome pathways (AOPs) can be identified and quantified by fluorescent protein reporters linked to specific receptors or enzymes. Spatial resolution of receptor or enzyme responses at the organ and tissue levels potentially enabling a much more accurate evaluation of subsequent pathological, physiological and functional responses. Temporal resolution is also possible in individual fish, enabling the identification of latent responses in organs and tissues. Quantification of MIEs and other key events and their calibration against adverse outcomes are crucial next steps for moving the application of AOPs from basic hazard assessment to intelligent risk assessment.

Using the “ERE-Casper” zebrafish as an illustrative model, we present a scheme for the application of transgenic zebrafish in chemical hazard and risk assessment and, at a more mechanistic level, show how they can serve to enable adverse outcome pathway analysis. The ERE-Casper zebrafish possesses a green fluorescent protein (GFP) reporter activated by estrogen response elements (ERE) and amplified via a transgenic promoter, making it highly sensitive to environmentally realistic exposure concentrations of selected estrogens (Lee et al., 2012). Furthermore, this model lacks skin pigmentation, making detection of the GFP response possible in both larval and juvenile life-stages and allowing for direct comparisons against adverse effects on development and function of targeted organs for these life-stages.

MO450
A newly designed transgenic zebrafish model to study the expression and the perturbation of corticosteroidogenesis by endocrine active substances at early developmental stages.

C. Garoche, M. Picot, INERIS; n. hinfray, INERIS / Ecotoxicology Unit; E. Chadili, INERIS; B. Piccinto, INERIS / Ecotoxicology Unit; C. Turès, INERIS; J. Porcher, INERIS / UMRI SEBIO; S. Ait-Aissa, INERIS / Ecotoxicology Unit; O. Kah, IRSET; E. Brion, INERIS / Ecotoxicology Unit

The 11β-hydroxylase (11βH) is the enzyme responsible for the biosynthesis of cortisol, the main corticosteroid in fish, which is implicated in multiple physiological processes including energy metabolism, hydromineral balance, reproduction and the immune system. In fish, the gene coding for 11βH, namely cyp11c1, is expressed early in intercellular cells during the development participating to the emergence of the corticosteroid axis. To further investigate the expression cyp11c1 and its potential deregulation by stressors and pharmaceutical ligands, we established a transgenic zebrafish line that expresses Green Fluorescent Protein (GFP) under the control of the zebrafish cyp11c1 promoter. Using in vivo fluorescence imaging to a high level whole larvae, we showed a weak expression of the reporter gene from 4 to 7 days post-fertilization (dpf) in an anatomical region where intercellular cells are known to be present. Tissue sections and immunostaining with specific 11βH antibodies revealed that GFP is co-localized with the endogenous cyp11c1 protein in intercellular cells. This cell agrees with the expression and localization of other steroidogenic interrenal marker genes such as star and cypl1a in wild-type zebrafish at this stage of development (To et al., 2007). To further characterize the cyp11c1-GFP zebrafish model, GFP was quantified in 6-dpf old zebrafish after osmotic stress or exposure to glucocorticoids receptor (GR) agonists (Dexamethasone, DEX) and antagonists (Mifepristone, MIF). Exposure to salt water led to a rapid induction of GFP, showing that cyp11c1 responds to an osmotic shock. Exposure to MIF (1µM from 5 to 6 dpf) decreases GFP intensity, suggesting that GR is involved in the transcriptional regulation of cyp11c1 during development. In DEX-exposed larvae (10µM, 5-dpf), GFP intensity was inhibited as compared to controls, this effect being abolished when zebrafish larvae were co-exposed with MIF. These data agree with the known negative feedback of DEX on pituitary expression of Pro-opiomelanocortin (POMC), leading in turn to a down-regulation of genes involved in corticosteroidogenesis (Sun et al., 2010). Altogether, these data suggest that the newly developed transgenic cyp11c1-GFP zebrafish is an interesting model to study the expression of 11β-hydroxylase gene in intercellular cells and its perturbation by endocrine active compounds acting on the Hypothalamic-Pituitary-Interrenal axis, which is critical for cortisol homeostasis.

Ecotoxicology of amphibians and reptiles. Resolving uncertainties about the impact of pollutants on individuals, populations and communities (P)

TU001
Pesticide risk assessment needed for amphibians and reptiles?

E. Steiss, EFSA / Pesticides Unit; A. Ippolito, EFSA - European Food Safety Authority / Institute for Environment and Sustainability IES; M. Arena, EFSA - European Food Safety Authority / Pesticides; D. Auteri, Auteri / Pesticides Unit; R. Sharp, EFSA - European Food Safety Authority / Pesticides Unit; C. Szentes, Pesticides Unit; J. Tarazona, EFSA

Many amphibians and reptile species are globally in decline and it is hypothesized that one of the causes for their decline is exposure to xenobiotic chemicals and complex interactions with other environmental stressors [1] [2] [3] [4]. Many amphibian and reptile species occur in agricultural landscapes and can be exposed to pesticides in the aquatic and the terrestrial environment. Exposure to pesticides could potentially lead to severe impacts on amphibians [5]. The EU regulation EU 1107/2009 requires for the approval of plant protection products no unacceptable effects on the environment, by considering particularly their impacts on non-target species including on the ongoing behaviour of those species and their impacts on biodiversity and the ecosystem. The data requirements for active substances (Regulation EU 283 / 2013) and formulations (Regulation EU 284 / 2013) ask for information on effects on terrestrial wildlife including amphibians and reptiles as well as for consideration of potential effects on non-target species. In order to enable a reliable assessment of the toxicity of aquatic life stages of amphibians which enabled a comparison of acute toxicity endpoints with other aquatic organisms. It is concluded in the aquatic guidance document [8] that the acute risk to aquatic life stages of amphibians is covered by the standard tier1 risk assessment for fish. It would need to be investigated further whether this covers also the risk to amphibians which develop in shallow non-permanent water bodies since exposure may be different than in the currently assessed FOCUS scenarios (i.e. permanent water bodies with a depth of 30 cm). Dermal exposure to pesticides is likely to be a major route of exposure for amphibians. In particular movements through treated fields could present a worst case exposure situation. EFSA has recently published a guidance document on non-dietary human exposure covering in-field and edge-of-field assessments, some elements could serve as a basis for future developments to address the dermal and inhalation exposure of other terrestrial vertebrates. In order to draw conclusions on what is covered by the current risk assessment more information on exposure estimates for shallow non-permanent water bodies, toxicity data (dermal and oral), feeding patterns and potential coverage of the risk by human non-dietary risk assessments is needed.

TU002
Challenges facing terrestrial amphibian risk assessment

J.M. Braunsch, BASF Corporation; P. Dohmen, BASF SE / Landwirtschaftsbereich APDR0

Risk assessment processes for agricultural chemicals are generally well developed for protecting aquatic species; however, there is currently no risk assessment.
Many uncertainties regarding exposure and effect remain, and need to be addressed in order to develop a comprehensive risk assessment procedure for terrestrial amphibians. This poster seeks to highlight some of these issues and to stimulate discussion of how they can be addressed moving forward. Terrestrial stage amphibian exposure to agricultural chemicals is highly variable and poorly understood. It is not clear to what extent amphibians can be exposed to agricultural compounds during routine applications, but many mitigating factors such as amphibian behavior, amphibian life-stage (metamorphs vs. adults), application timing, crop stage, etc. can substantially alter exposure. These factors are critical to understand, and are vital for producing a realistic exposure framework. In addition to the uncertainties regarding exposure to agricultural compounds, limited information is available on the effect of pesticide exposure on the reproductive behavior and fitness of amphibians. The reproductive fitness of amphibians is considered one of the most important endpoints for risk assessment, and exposure of amphibians to agricultural chemicals may decrease fertility or survival of breeding individuals, and alter the population dynamics. Despite this, there are few studies that evaluate the effects of pesticides on amphibian reproduction. In this study we evaluated the presence of a single pesticide application during metamorphosis on juvenile survival, growth and development of Rana pipiens. Our results show that exposure to a single application did not significantly affect juvenile survival, growth or development. However, additional studies are needed to evaluate the long-term effects of pesticide exposure on amphibian reproduction.
study was to evaluate differences in the area/volume ratio of mucous glands of natural populations of *Pelophylyx perezi* exposed to agropesticides within contaminated sites. Seven adult females from four sampling areas were collected. Adults were euthanized with MS222. Morphometric parameters were determined and skin was removed for posterior histological analysis. Pieces of skin were fixed in Bouin solution and then processed for paraffin-wax embedding using routine protocols. Subsequently, sections were cut and stained with several colorations (e.g. H&E, Alcian Blue and PAS). Sections were analyzed at the fluorescence microscope and the measure of each gland was carried out to observe differences between sites. Preliminary data analysis shows that there is a significant increase (p < 0.05) in gland area ratio in organisms from a contaminated area when compared with the control site. Keywords: Agopicidestics, histology analysis, mucous glands, oseomurulation, P. perezi.

TU008

Exposure of spotted salamander (Ambystoma maculatum) egg masses to a PSII inhibitor throughout embryonic development

L.R. Baxter, University of Guelph / School for Environmental Science; R. Brain, Syngenta Crop Protection, Inc. / Department of Environmental Risk Characterization; A.J. Hosmer, Syngenta Crop Protection, Inc.; K.R. Solomon, University of Guelph / School of Environmental Sciences; M.L. Hanson, University of Manitoba / Department of Environment and Geography

The North American yellow-spotted salamander (*Ambystoma maculatum*) produces gelataneous egg masses that are colonized by a green algae (Chloraophyta) commonly referred to as *Oophila ofsp.*. This has been reported as a mutualistic relationship, indicating that embryos may be indirectly impacted if algal growth is reduced. Since the wide distribution of this salamander includes major agricultural regions, the question of how herbicides might impair this relationship has been raised. After previously assessing sensitivity of *Oophila sp.* in laboratory cultures to the PSII-inhibitor atrazine, we examined impacts of this herbicide on algal growth and tadpole development. The primary goal of this study was to examine hatching success, size and stage of *A. maculatum* larvae at hatching following exposure to atrazine throughout embryonic development. *A. maculatum* egg masses were collected in Algonquin Provincial Park, Ontario, Canada in May 2014, and exposed to technical-grade atrazine at nominal concentrations of 0, 3, 10, 30, 100 and 300 µg/L (n=5 for controls; n=3 for treatments) until hatching was complete (up to 66 d). Controls (untreated) and exposures to atrazine occurred in a controlled environmental chamber under field-like conditions of light and aeration. Responses were measured simultaneously in untreated egg masses in 24-h dark with and without aeration, and in the light without aeration (n=3). In addition, algae was isolated from a reserved egg mass in a controlled laboratory inhibition of growth by 96-h exposure and recovery periods. No statistically significant differences in stage at hatching were observed for any treatment (*Kruskal-Wallis ANOVA* on ranks). Treatment with atrazine did not significantly affect average days to hatch, length at hatch or hatching success (*MANOVA; F₁₇,₁ = 1.02, p = 0.455). In toxicity testing of cultured algae, responses were similar to those observed previously, with an EC₅₀ value of 16.4 µg/L. *O. ofsp.* on *A. maculatum* embryos as measured in this study was not impacted by exposure of egg masses to technical-grade atrazine at concentrations and durations exceeding those commonly found in the environment. Growth of the algae *Oophila sp.* appears to be less sensitive to atrazine in the natural egg environment than in laboratory culture.

TU009

Differential and short-term effects of three maize herbicides (linuron, S-metolachlor, dicamba) to Perez’s frog embryos

V. Galhano, Department of Biology & CESAM - University of Aveiro / Biology dBio; C. Quintanese; M.S. Monteiro, Aveiro University / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM

In Portugal, many surface waters and groundwater are vulnerable to anthropogenic contamination or environmental variables can potentially alter the chemical toxicokinetics and therefore one current challenge is to predict the risks established by the interactions between nonchemical and chemical stressors at biological level. In Brazil, many species of amphibians are distributed in common agriculture areas and they mainly breed during the rainy season, which is the period of greatest environmental pesticides contamination. Sulflurazone is a potent herbicide used on crops of sugar cane and soy in the southeastern Brazil with a long residual effect in the soil and high potential for contamination. In this study, we evaluated the effects of temperature on the toxicity of sulflurazone (commercial formulation Bora®80SC) in tadpoles of *Euphlyophila perezi* by analysis of oxidative stress biomarkers. Tadpoles at stage 29–32 were exposed to 0.01, 0.05 and 0.1 mg/L to temperatures of 28°C, 32°C, and 35°C during 3 and 8 days. Results from antioxidant enzymes, s, show differences between treatments. At 32°C and 35°C after 8 days, glutathione-S-transferase (GST) activity decreased at concentrations of 28°C and 32°C after 3 days. After 8 days, GST was induced by sulflurazone concentrations at 32°C and 35°C. Activity of glucose-6-phosphate dehydrogenase (G6PDH) was increased by all concentrations at 32°C and 36°C in both experimental periods. Lipid peroxidation (MDA) was also induced by the treatments. MDA increased in animals exposed to 0.5 and 0.1 mg/L at 28°C and 32°C after 3 days. Exposure to 0.01 and 0.1 mg/L also increased MDA up to 4 days in 36°C. The comparison among control groups also showed that tadpoles maintained in 36°C had the highest antioxidant enzymes activities and MDA levels.

The results show that temperature is an important environmental stressor influencing the toxicity of sulfurazone in tadpoles and the changes in oxidative stress biomarkers are probably a result of physiological adaptation to stress conditions established by the interaction between chemicals and different temperatures.

TU010

Effects of temperature on the toxicity of sulflurazone in tadpoles of *Euphlyophila perezi* (Leptodactylidae): oxidative stress biomarkers

J.S. Freitas, Chemistry; E.A. Almeida, Chemistry and Environmental Sciences

The increase in temperature and other environmental effects influenced by global climate changes (GCC) have documented several impacts on global amphibian populations. Changes in environmental variables can potentially alter the chemical toxicokinetics and therefore one current challenge is to predict the risks established by the interactions between nonchemical and chemical stressors at biological level. In Brazil, many species of amphibians are distributed in common agriculture areas and they mainly breed during the rainy season, which is the period of greatest environmental pesticides contamination. Sulflurazone is a potent herbicide used on crops of sugar cane and soy in the southeastern Brazil with a long residual effect in the soil and high potential for contamination. In this study, we evaluated the effects of temperature on the toxicity of sulflurazone (commercial formulation Bora®80SC) in tadpoles of *Euphlyophila perezi* by analysis of oxidative stress biomarkers. Tadpoles at stage 29–32 were exposed to 0.01, 0.05 and 0.1 mg/L to temperatures of 28°C, 32°C, and 35°C during 3 and 8 days. Results from antioxidant enzymes, s, show differences between treatments. At 32°C and 35°C after 8 days, glutathione-S-transferase (GST) activity decreased at concentrations of 28°C and 32°C after 3 days. After 8 days, GST was induced by sulflurazone concentrations at 32°C and 35°C. Activity of glucose-6-phosphate dehydrogenase (G6PDH) was increased by all concentrations at 32°C and 36°C in both experimental periods. Lipid peroxidation (MDA) was also induced by the treatments. MDA increased in animals exposed to 0.5 and 0.1 mg/L at 28°C and 32°C after 3 days. Exposure to 0.01 and 0.1 mg/L also increased MDA up to 4 days in 36°C. The comparison among control groups also showed that tadpoles maintained in 36°C had the highest antioxidant enzymes activities and MDA levels.

The results show that temperature is an important environmental stressor influencing the toxicity of sulfu-razone in tadpoles and the changes in oxidative stress biomarkers are probably a result of physiological adaptation to stress conditions established by the interaction between chemicals and different temperatures.

TU011

The oral toxicity of several rodenticides and herbicides to the western fence lizard (*Sceloporus occidentalis*)

S.M. Weis, Savannah River Ecology Laboratory / Savannah River Ecology Laboratory; S. Yu, University of Guelph / School of Environmental Toxicology; J. Monks, New Zealand Department of Conservation; L.G. Talent, Oklahoma State University; C.J. Salice, Towson University / Biological Sciences Environmental Science

The effect of invasive species on island ecosystems has been well established. On the islands of New Zealand invasive mammals have had detrimental effects on native species including the western fence lizard (*Sceloporus occidentalis*). Pesticides have played an important role in the control of invasive species, but the use of pesticides may itself affect native species. Here we provide oral toxicity data for 5 rodenticides and 5 herbicides used in New Zealand to the western fence lizard (*Sceloporus occidentalis*) as a surrogate for native New Zealand reptiles. We quantified toxicity using a standard up-and-down method (UDM) with few minor changes. Reptiles were observed for 14 days following single oral doses. Doses followed the standard UDM guidelines: 5.5, 17.5, 55, 175, 550 and 1750 µg/g. Reptiles were dosed with pure pesticides using gelatin capsules, no carrier solvents were used. Only one rodenticide (pindone, LD₅₀ = 550 µg/g) and one herbicide (triclopyr, LD₅₀ = 550 µg/g) were found to be toxic to reptiles at doses tested. The remaining compounds were not toxic up to 1750 µg/g. Our data support the potential risk of these pesticides to native reptiles. We have not yet investigated the effects of formulated products, or the likelihood of exposure under natural scenarios. Understanding potential pesticide exposure as well as the toxicity of formulations is an important next step to understanding risk of these pesticides to reptiles.

TU012

Effect of cadmium contaminated soils on reproductive fitness and offspring survival of the wall lizard *Podarcis sicula*

R. Scadiero, Department of Biology; P. Simonelli, GSI Helmlholtz Center for Heavy Ion Research / Department of Biophysics; F. Trinchella, C.M. Motta, University Federico II of Naples / Department of Biology

Animal species are under extinction risk due to several factors (habitat loss, global warming, introduction of exotic species, emerging pathologies). Environmental pollution is one of the most relevant extinction causes for animals such as amphibians, while terrestrial oviparous vertebrates are considered more resistant to soil contaminants. Their embryos are considered well protected by shells from the
external environment; the presence of contaminants in eggs or developing embryos is attributed to maternal transfer during vitellogenesis and oviductal egg retention. Recently, it has been demonstrated that metals present in soil may cross the parchment-like shell of reptilian eggs. So, we decided to investigate cadmium effects on reproduction and development in *Podarcis siculus*, a lizard inhabiting both pristine and urbanized areas. Our data demonstrate that both adults and embryos are highly susceptible to Cd adverse effects and that Cd accumulation, possibly through non-lipid transfer via food chains, gives rise to the accumulation of the metal in tissues. The accumulation in liver is accompanied by severe damage at multiple levels interfering with tissue structure and function, in ovary is accompanied by stimulation of oogonial proliferation, oocyte recruitment and follicular atresia. The effects exerted by Cd in Podarcis ovary are not typically estrogenic as expected but rather FSH-like, and strongly reduce fecundity and reproductive performance. In embryos, incubation in Cd contaminated soil causes damage to forebrain vessels and eyes. In addition, Cd is able to interfere with gene regulation, affecting the expression of genes associated with pathways such as membrane trafficking, signal transduction, neuronal transmission and regulation of gene transcription. The dysregulation in the expression of these genes could explain many of the morphological alterations displayed by embryos. Although no in ovo mortality was observed, the severe malformations may be incompatible with adult survival. Since almost all reptilian species lay their eggs in subterranean nests, the rapid decline in many reptilian populations observed in recent years might be partly ascribed to developmental failures in offspring in the increasingly polluted environments, and partly ascribed to the impaired fecundity of Cd contaminated females, leading to a level of malformations and a linear decline in the number of individuals for wild populations living in polluted areas.

TU013 Does thermal stress affect lizard embryonic development in the same way of chemical stress? R. Scudiero, Department of Biology; M.G. Esposito, University Federico II of Naples; P. Simonello, GSI Helmholtz Center for Heavy Ion Research / Department of Biophysics; C.M. Motta, University Federico II of Naples / Department of Biology

Species distribution across geographic regions is generally controlled by environmental temperature, especially when the animals are ectothermic. The current models of climate change predict the increase of average global temperature and the frequency and intensity of periods with extreme temperatures. These changes may have pronounced effects on ectothermic species capacities, survival and distribution. Growing evidence demonstrates that environmental temperature is a particularly important factor in determining developmental rates, morphological alterations and performance of ectotherms as well as fetal morphology of newborn organisms. In reptiles, temperatures experienced by developing embryos determine the species’ distribution as well as its ability to hatch and survive. We examined the effects of thermal stress (both cold and warm stresses) on the development of the oviparous lizard *Podarcis siculus*. The results showed a severe negative impact of constant thermal stress on the viability of embryos, resulting in lethality, reduced survivorship and abnormal development. A preliminary analysis on gene expression demonstrated changes in the embryos transcriptome in response to cold shock. These findings demonstrate that lizard embryos show a very narrow temperature ranges and that thermal stress during embryonic development may be a crucial factor for affecting adverse effects. This higher sensitivity of embryos also suggests that many lizard populations will be most affected by global climate change, with severe reductions in hatching production. Interestingly, similar morphological alterations (in particular, anencephaly, brain and eye malformations), such as the dysregulation of gene expression were observed even when *P. siculus* embryos were incubated in cadmium contaminated soils. Thus, we can speculate that in *P. siculus*, and possibly in many other organisms, different environmental stressors bring comparable effects both at morphological and molecular levels. More studies are needed to elucidate the potential harm of the interactions between temperature and cadmium on the developmental stages of ectothermic vertebrate species and to reach to an accurate prediction of pollutants toxicity, taking into account other variables that act in concert with the toxicants.

TU014 Effects of cadmium on maturation, fertilization and development of Xenopus laevis oocyte L. Lagier, ECOLAB UMR 5245 CNRS UPS INPT / Ecotoxicology and Environmental Health; A. Lescuyer, University of Lille / Lille Sciences and Technologies Equipe Régulation des Signaux de Division UGFR UMR; C. Sarrieu, ECOLAB UMR 5245 CNRS UPS INPT / Ecotoxicology and Environmental Health

Cadmium (CdCl₂), an inorganic compound with natural and mainly anthropogenic sources, such as metallurgy, was chosen. We first focused on cell survival with phenotypical (morphology, pigmentation), physiological (maturation ability) and electrophysiological (resting potential) approaches. Then, maturation was induced in vitro, using morphological migration and break down of the germinal vesicle – GVBD, biochemical (activation of MAPK – Mitogen Activated Protein Kinase – and MPF – Phase Promoting Factor – pathways) and histological (meiotic spindle formation, polar body extrusion) assays. Finally, fertilization and early stages of development (up to one week) were studied. In an attempt to understand the role of Cd in the oocyte maturation process, we showed that chlorate metal exposure affects oocyte survival with both morphological and electrophysiological methods. Here, oocyte survival was considered by a new physiological method: the ability to undergo maturation after hormonal (progesterone) stimulation i.e. the gain for the oocytes to be competent for fertilization. In a second set of experiments, after cadmium exposures, we demonstrated that MPF pathway (associated with oocyte maturation) is deregulated. Fertilization was then assessed when male and female gametes were exposed, either separately or together, to Cd. Our data showed that cadmium exposure was responsible for adverse effects in a dose dependent manner on in vitro fertilization rates, development and organogenesis. In addition, our results showed that cadmium preferentially targeted the female gamete rather than spermatozoon, and prevented them to be fertilized. Taken together, our results demonstrated that early stages of amphibians could be critical windows of exposure to cadmium.

TU015 Combined toxicity of zinc, copper and cadmium on the tadpoles *Lithobates catesbeianus*: acute and chronic effects on liver antioxidant system M.N. Fernandes, Universidade Federal de Sao Carlos / Ciencias Fisiologicas; H. Utsomiyomiya, Universidade Federal de Sao Carlos; T. Passuqueto, Universidade Federal de Sao Carlos; M.J. Costa, Federal University of Sao Carlos (UFSCar) - Sororoca / Department of Biology; M.M. Sakuragi, Federal University of Sao Carlos / Department of Physiological Ciencias; C.S. Carvalho, Universidade Federal de Sao Carlos

The effect of metals (Zn+Cu, Cd+Cu and Zn+Cu+Cd, 1 µg L⁻¹) on the antioxidant system in the liver of *Lithobates catesbeianus* was analyzed after for 2 and 16 days exposure. After 2 days, all metals increased in the liver (50-300%) and after 16 days exposure, the concentration of Zn (240%), Cu (138%), Cd (60%), and in all groups associated to Zn (100–430%) increased. Cd concentration decreased when associated to Zn and Cu (20-80%). After 16 days to exposure to isolated metals, Cd accumulation decreased, Cu and Cd+Cu increased (50-90%) but groups increased in exposure to Cu (185%), Zn+Cu (100%) and Zn+Cd (120%). The activities of SOD, CAT, GPX and GST increased in most groups after 2 days exposure to isolated and associated metals. After 16 days exposure, the activities of SOD, CAT and GST decreased and GPx increased. The enzymes activity increased in the controls after 16 days compared to 2 days exposure and, SOD, CAT and GPx increased after 16 days in relation to 2 days. However, GST activity decreased after 16 days exposure to Cu (60%), Cd (70%), Zn+Cu (60%), Zn+Cd (50%), Cu+Cd (57%) and Zn+Cu+Cd (35%). The levels of MT and GSH decreased after 2 days, but increased after 16 days. LPO levels increased after 2 days and decreased after 16 days. The levels of GSH and LPO decreased in controls after 16 days of exposure compared to 2 days, and MT levels decreased in fish exposed to Cu (54%) and Cd+Cu (54%) but increased in fish exposed to Cu (87%). GSH levels increased in fish exposed to Cu (38%), Zn+Cu (106%), Zn+Cd (144%) and Zn+Cu+Cd (61%). LPO levels decreased in all groups exposed to isolated and associated metals after 16 days compared to 2 days. The high levels of metals in the liver of tadpoles demonstrated that, although the metal concentrations in the present study are below the maximum concentration considered environmental safe to the Brazilian Environmental Council (CONAMA, res 350/2005), they cause acute or chronic effects on bullfrog tadpoles. The process of metal uptake is influenced by several factors, such as their chemical form in water (soluble or particulate), the physical and chemical conditions of the aquatic environment (temperature, pH, salinity, dissolved O₂), and the animal physiological condition (stage development, age and size, activity period of the reproductive cycle). The antioxidant system showed protects liver against oxidative stress. Financial support: FAPESP Proc. 2011/50752-3.

TU016 Ecotoxicity of carbon-based nanoparticles in Xenopus laevis larvae M. A. V. Nascimento, Univerdidade Federal do Espirito Santo / Ecotoxicology and Environmental Health; J. F. R. Ataíde, Univerdidade Federal do Espirito Santo / Ecotoxicology and Environmental Health; F. Caetano, CIRIMAT/NAUTILE; M. J. Costa, Federal University of Sao Carlos (UFSCar) / Sororoca / Department of Biology; M. M. Sakuragi, Federal University of Sao Carlos / Department of Physiological Ciencias; C. S. Carvalho, Universidade Federal de Sao Carlos

Because of their unique properties, carbon-based nanoparticles (CNPs) such as multi-walled nanotubes (MWNT) and double-walled nanotubes (DWNT), few layer graphene (FLG) and graphene oxide (GO) are or will be widely produced and used around the world. Faced with this increasing development, anticipates environmental risks associated with these emerging contaminants is a necessity. Financial support: FAPESP Proc. 2011/50752-3.
Aquatic environment may be particularly affected by the release and the transfer of these materials. However, few ecotoxicological data are currently available. The objective of the present work is to assess the ecotoxicity of well-characterized CNPs on amphipathic larvae ( Xenopus laevis ) using standardized tests ( ISO, 2006 ) [1]. The exposure concentrations ranged from 0.1 to 50 mg/L. Various endpoints were studied, i.e. (i) acute toxicity (mortality), (ii) chronic toxicity (growth inhibition), and (iii) genotoxicity (induction of micronucleated erythrocytes). Moreover, the effects of CNPs were compared with those of each other type of CNPs. No mortality was recorded for all CNPs whatever the concentration. By contrast, significant growth inhibition was observed from 10 mg/L of DW and at 50 mg/L of MW and GO. No growth inhibition was observed for FLG. The chronic toxicity observed in larvae exposed to high concentrations of CNPs could be limited to physical effects (gill obstruction, and/or abrasive effects and or nutrients deprivation). Genotoxicity has been only observed in larvae exposed to GO. This work has been carried out in the frame of the European FP7 FET program Graphene Flagship and the French joint laboratory NAUTILE. [1] ISO. 2006. ISO International Standard. Water quality - Evaluation of genotoxicity by measurement of the induction of micronuclei - Part 1: Evaluation of genotoxicity using amphipathic larvae. ISO 21427-1, ICS: 13.060.70, Genova - CH.


In Canada, the Federal Contaminated Sites Action Plan (FCSAP) supports site managers in reducing human health risks, ecological risks and financial liabilities associated with federal contaminated sites. FCSAP provides guidance for ecological risk assessment that promotes applying a comprehensive weight of evidence approach to assess risk from contaminants to all receptors, including amphibians. Risk assessment research was undertaken to develop a method that can (i) assess contaminated sites through different lines of evidence: 1) conducting site-specific toxicity tests; 2) comparing exposure at the contaminated site to literature-based toxicity data; 3) conducting site-specific biological field studies; and 4) comparing site-specific exposure to biological field studies reported in the scientific literature (FCMPS 2012). Until recently, amphibians have often been excluded from risk assessments because site-specific methods or literature data are either not available or not easily accessible. In order to facilitate risk assessments for amphibians, Environment Canada is developing practical guidance for amphibian risk assessments for the four different lines of evidence. For site-specific toxicity and biological testing, available methods are being evaluated and provided to the risk assessor. This method compares site-specific contaminant concentrations to concentrations in laboratory-based toxicity studies, amphibian toxicity concentration-response data is being compiled for metals that are particularly toxic to amphibians (Birge 1978). The toxicity data covering multiple endpoints is illustrated as a multi-species concentration-response relationship. This allows the risk assessor to go beyond point-estimate based hazard quotients and evaluate risk in context of effect magnitude and uncertainty across a range of concentrations present at federal contaminated sites. The concentration-response data compilations also confirm whether contaminated sites water quality guidelines, which are typically developed without specific amphibian considerations, are providing adequate protection for this sensitive group of receptors.

TU018 Interpreting Biological Effects of Metals and Their Mixtures (P)


Sediment in aquatic environments acts as a major sink of metal contaminants and has the potential to be released into the water column through dredging, changes of physicochemical conditions and feeding and burrowing activities of benthic biota, leading to continuous contamination of aquatic systems. To evaluate fate and effects of sediment bound metals, sediment toxicity tests are increasingly being used in laboratory and field conditions. The exposure-dose-response (EDR) approach has been proposed as prognostic tool in sediment toxicology studies as it integrates the chemistry of abiotic matrices with bioaccumulation and impairment of biological systems. In this study, the freshwater bivalve Hyriidea australis was selected as sentinel organism to use within this frame work. Previous studies have used EDR antimicrobials for the evaluation of silver concentrations in its under laboratory conditions. In the environment metals are present as mixtures, therefore, toxic effects are not a result of a single metal exposure, but is rather a results of exposure to mixtures of metals due and their toxicological interactions. In this context, exposure-dose-response relationships of H. australis to a mixed metal contaminated sediment was investigated. This study is being conducted in parallel to other cage based experiments in the Molonglo River to further understand differences of cause and effects of the same mixed metal contaminated sediments under environmentally realistic and laboratory conditions. H. australis were exposed for 28 days to four different combinations of mixed metal contaminated sediments collected from the bivalve caged sites at the Molonglo River. After 28 days of exposure, metal dose was measured as in whole body and individual tissues. Sub-cellular analysis is conducted in hepatopancreas tissues to examine the biologically available and detoxified metal content. Enzymatic and cellular biomarkers are measured as responses to metal toxicity in hepatopancreas tissues.

TU020 The effect of waterborne silver on survival, ionoregulatory function, metallothionein and nks 2.5 inductions in zebrafish (Danio rerio) embryos S.M. Bakir, Plymouth University / Biological Sci; R.D. Handy, Ecotoxicology Research and Innovation Centre; T.B. Henry, Heriot-Watt University / School of Life Sciences

Natural leaching from silver sites and anthropogenic activities, as well as Ag-NPs that are used EDR antimicrobials, lead to elevation of silver concentrations in its aquatic environment. Silver salts such as AgNO3 are soluble and dissociates to yield free silver ions (Ag+) which are known to cause ionoregulatory toxicity in adult fishes. However, the effects on fish embryos and their development are less well understood. The objectives of this study were to investigate the effects of dissolved silver on the survival and hatching success of zebrafishes, and then to understand the sublethal responses that relate to defences against toxic metals (metallothionein expression), osmoregulatory disturbance (Na/K-ATPase, electrolyte levels), oxidative stress(total glutathione), and cardiac development (nk2.5 gene). Embryos < 1hpf were exposed to silver 0 (no added Ag), 2.5, 5, 7.5, 10, and 15 μg 1 Ag as AgNO3 for up to 72 h. Although, the survival was not affected by increasing concentrations of total silver, a decrease in hatching and increase in heart beat was observed (ANOVA, p < 0.05). Live and dead embryos were collected at 24 and 72 hpf for silver determination and biochemistry. A significant (ANOVA p < 0.05) increase in embryonic silver accumulation in both live 24 and 72 hpf and dead embryos, also it was observed more significant accumulation (two way ANOVA, p < 0.05) in 24 hpf live embryos. Dead embryos and live embryos at 72 h exposed to AgNO3 25 μg/L and 50 μg/L NKA, p < 0.05). Embryos also showed a transient increase in Cyt C concentration at 24 h (two way ANOVA, p < 0.05). At 4, 4, 8, and 16 fold increase (two way ANOVA, p < 0.05) in “K+”-ATPase activity, Mt2, total glutathione, and protein concentration respectively were seen in embryos after 72 h of exposure to AgNO3 compared to controls. In contrast, nk2.5 gene expression significantly decreased (two way ANOVA, p < 0.05). Embryos exposed to silver compared to controls. Silver toxicity is consistent with ionoregulatory toxicant in embryos that also effects gene involved in development.
compounds. Therefore, it is important to investigate the toxicity of metals when present in a mixture. Mixtures of dissolved metals can exhibit a different (higher or lower) toxicity than predicted based on the toxicity of the individual metals. Such a deviation from additive may, however, become less pronounced when more metals are added to the mixture. Therefore, this study aimed to analyze the effect of increasing numbers of metals in a mixture on the toxicity to Daphnia magna. To this end, we have performed a series of metal mixture toxicity tests according to OECD guideline nr. 202. The selected metals were Ni, Cd, Zn and Cu, tested individually and in mixtures. Binary, tertiary and quaternary equitoxic mixtures were composed using the Toxic Unit (TU) concept. There were 5 concentrations per test, 4 replicates per concentration and 5 daphnids per replicate. EC50 values and the corresponding 95% confidence limits were calculated applying a logistic response model. The results showed significant more-than-additive as well as less-than-additive effects of the metals in binary mixtures. When the number of metals in the mixture was increased from two to three, deviations from additivity decreased. No deviation from additivity was observed when the mixture consisted of four metals. Thus, decreased deviation from additivity was observed in a mixture of three metals, which remained apparent in the mixture of four metals. It is therefore concluded that in complexly polluted ecosystems, where mixtures with even higher numbers of metals are typically present, concentration additivity might be the rule rather than the exception. Keywords: complex metal mixtures; concentration addition; aquatic ecotoxicity, Daphnia magna

TU022
Mixture toxicity of zinc and copper on the growth of the diatom Navicula pelliculosa and comparisons with concentration additive and independent action predictions
T. Nagai, National Institute for Agro-Environmental Sciences; K.A. De Schamphelaere, Ghent University (UGent) / Laboratory for Environmental Toxicology and Aquatic Ecology GHelToxLab unit
Metal mixture effects on algal growth have not often been investigated, especially for species other than green algae. Moreover, the applicability of conventional mixture toxicity prediction models, concentration additive (CA) and independent action (IA), has also not sufficiently been investigated. Here, we investigated the mixture toxicity of zinc and copper on the growth of a diatom, Navicula pelliculosa. A toxicity test was conducted with 96 Daphnia magna microplates for 72 h, and the algal growth was monitored using a fluorescence microplate reader. A 7x7 full factorial experimental design was used for the mixture test (49 combinations) in total. Tested concentrations were EC1/4, EC1/2, EC1, EC3/2, EC3, EC5, and EC7, which were determined by preliminary single metal toxicity tests. Free ion activities in test solutions were calculated using chemical equilibrium speciation software Visual Minteq (v3.0.1). The predicted free metal ion activity-response relationships of both single metals. CA prediction was conservative at all level and IA was a better predictor than CA. However, the deviation of IA predictions from experimental results increased with increasing effect levels. Synergistic effects relative to IA were observed at higher effect levels. These results suggest that the toxic mode of action of metal mixtures may vary depending on the effect level.

TU023
Evaluation of chronic Interspecies Correlation Extrapolation (ICE) models using metals
D. Leverett, A. Peters, Wea Environment Ltd.
Interspecies Correlation Models (ICE) allow the prediction of toxicity of a substance to an untested species based on the known toxicity of the substance to a single surrogate species. ICE models are generally log-linear least squares regressions based on the known toxicities of two species to a range of different substances. The regression of the differential toxicity for the two species therefore allows an extrapolation of the toxicity for a substance where only one of the two species has been tested. The USEPA has developed a series of ICE databases (covering aquatic animals, algae and wildlife) which utilises approximately 2400 models to predict toxicity, and which also includes extrapolations for Species Sensitivity Distributions (SSDs). However, the USEPA models are all based on acute toxicity endpoints, and the technique is therefore of limited use in assisting in investigating chronic exposure to the chronic SSDs. Under the majority of EU chemicals regulations (e.g. WFD), toxicity results (NOEC/ EC10s) covering a minimum of 10 species are required before an SSD approach can be considered for the derivation of long-term quality values. For substances with smaller datasets, a deterministic approach is therefore the only option in deriving such standards, with associated large assessment factors. In this poster we present an initial attempt at developing ICE for chronic endpoints for invertebrates, algae/ plants and fish using the large chronic aquatic toxicity databases which are available for some metals (e.g. zinc, copper, nickel, and silver), and discuss the potential for this approach to be utilised in deriving estimates for chronic toxicity to untested species in order to supplement chronic toxicity datasets, and to assist in developing SSDs for other, less data rich, metals.

TU024
INFLUENCE OF pH AND DISSOLVED ORGANIC CARBON CONCENTRATION ON URANIUM TOXICITY TO DAPHNIA MAGNA: IMPLICATIONS FOR ACID MINE DRAINAGE TREATMENT AND CATCHMENT PROTECTIVE WATER QUALITY STANDARDS
S.K. Kahule, USK Consulting / ENVIRONMENT WASTE ENGINEERING; S.J. Klaine, Clemson University / Institute of Environmental Toxicology CUENTOX
In an earlier study, pH variations due to acid mine drainage and organic matter loading from point source discharges of domestic wastewater treatment plants, appeared to dictate the chronic toxicity profile of the Wonderfulfont Spruit, a mine tailings dam located in the West Rand Gold Basin of South Africa. In this catchment where dissolved Uranium concentrations range between 20-250 μg/L, both acute and chronic toxicities to D. magna, in this catchment were attributable todissolved uranium concentrations alone. A significant reduction in toxicity was observed at sites located just downstream of wastewater treatment discharge into the catchment. DOC concentrations increased at sites downstream of the wastewater discharges into the river, and this resulted in significant reductions in both survival and reproduction of D. magna. These results suggest that stream segments downstream of WWTW discharges were healthier and that the reason was the increase in pH and dissolved organic carbon in the stream. In this paper follow-on study, the influence of dissolve organic carbon (DOC) and pH on the toxicity of U to D. magna was assessed using a multifactorial test design. Exposure tests were conducted in moderately hard water reconstituted with pH (4.5 - 9.5), DOC (0 - 10 μg/L) in the form of natural organic matter (NOM) standard Suwannee River Fulvic Acid (FA). Organisms were exposed to a range of U concentrations ranging from (1.375 - 22 mg/L). Results revealed that DOC concentration and pH had a significant effect on Uranium toxicity. In general, an increase in pH and DOC resulted in a decrease in toxicity. For example, at pH 7 and 10 mg/L DOC, the EC50 was significantly less in tests with 10 mg/L DOC, relative to tests with 0 and 2 mg/L DOC. Tests also revealed that DOC concentration was the most important factor in influencing effects of Uranium toxicity to D. magna, explaining above 64% of the observed effects as compared to pH. Speciation predictions of Uranium in moderately hard water performed using Visual Minteq could also explain some of these results. When the model output accounted for reduction in toxicity due to complexing of uranyl species, such as UO2+2, UO2+H+ and other positively charged species by DOC. These results could be used to predict concentration of DOC and pH that would significantly reduce toxicity in a system. Further this research can become a first simple tool for establishing biotic ligand model derived catchment protective water quality standards.

TU025
Nickel toxicity in soft waters
Nickel toxicity in soft waters. The lower validation limit of the European nickel BLMs is at a calcium concentration of 2 mg L−1. However, a potentially significant proportion of Australian waters show water hardness below this value. Previous acute and chronic toxicity modelling studies with Ni indicate that soft water organisms exhibit an increased protective effect of calcium and magnesium on nickel toxicity than is observed in hard water organisms. This study aims to establish the degree of competition from calcium and magnesium in calculating the accumulation of nickel at a hypothetical “biotic ligand” in an Australian invertebrate species which is able to tolerate very soft water chemistry conditions. Chronic nickel toxicity testing on Hyalea viridisilissa was performed in a very soft water, which will be amended with increasing concentrations of calcium and magnesium to identify their effect of nickel toxicity. The findings of the study will be used to refine the application of bioavailability models for nickel in soft Australian waters.

TU026
ARSENIC TOXICITY ISOLATED AND ASSOCIATED WITH IRON TO THE TROPICAL CLADOCERAN Ceriodaphnia silvestrii
S.C. Sales, Federal University of Minas Gerais / Department of Zootechnia; A.C. Rietzler, M.M. Martins Ribeiro, Department of General Biology
Arsenic is an ametal widely distributed, and its occurrence is associated with oxides and hydroxides of iron and manganese or organic matter. Evaluating arsenic toxicity to aquatic organisms is critical to preserve these ecosystems and to prevent human health concerns. Chronic As toxicity can be considered as an essential component in transferring metals through the trophic chain being used in ecotoxicological studies. The aim of this study was to evaluate arsenic toxicity isolated and associated with iron to Ceriodaphnia silvestrii. Three experiments were conducted: (1) evaluation of arsenate and arsenite EC50; (2) evaluation of arsenate IC25, considering surface waters containing predominantly the pentavalent form; and (3) evaluate arsenate EC50, considering the presence of Fe (III). Among the results, the EC50 ranged between 0.69 mg L−1 (As V) and 0.44 mg L−1 (As III). Furthermore, in the presence of iron at concentrations 0.02 e 2.00 mg L−1 EC50 was significantly reduced (0.37 mg L−1) demonstrating a synergistic effect. However, the IC25 mean value was 0.59 mg L−1 (As V), indicating detoxification mechanisms reducing arsenic toxicity. The
arsenite showed higher toxicity than its pentavalent form, but this variation was lower than expected from the literature. The toxicity of arsenic significantly increased associated with iron. These results demonstrated the need for monitoring and reduction of arsenic in aquatic environments, especially in places where the geological matrix is composed of iron ore.

TU027
Effect of copper contamination on Tribolium castaneum: large differences in sensitivity between life history traits
A.J. Bednarska, Jagiellonian University / Institute of Environmental Sciences; R. Laskowski, Jagiellonian University / Ecotoxicology Stress Ecology Group; W. Makarowski, K. Stepień, P. Gibas, Jagiellonian University / Institute of Environmental Sciences

Effects of toxicants on animals are most frequently evaluated in short term experiments with mortality as the only endpoint. It is known, however that sensitivity of particular life history traits to toxicants can differ, and prolonged exposure may cause delayed effects. Moreover, detailed quantitative information about effects of toxicants on individual life-history traits is necessary for better understanding and mechanistic explanation of underlying processes. This is particularly important for holometabolic insects with their complex lifecycles and a number of life stages, each of which can differ in sensitivity to toxicants. To compare sensitivity to a toxicant of all life history traits of a holometabolic insect we performed an experiment on the red flour beetle Tribolium castaneum (Coleoptera: Tenebrionidae) exposed to Cu at 0, 1000, 2000 or 4000 mg/kg for 90 days. The following life history traits were recorded: hatching, growth rate, duration of larval and pupal stages, larvae and pupae size, adult emergence, sex ratio, adult body mass, fecundity (in randomly mated pairs) and survival. Each individual was followed separately from the egg stage. Larvae survival was high in all treatments with no significant Cu effect, even if the highest survival, 96%, was found in control and the lowest, 80%, in the highest Cu treatment. The growth rate of T. castaneum larvae was best described by the logistic function, with body mass increasing exponentially at the beginning and levelling-out before the pupation. The growth rate at the two highest concentrations was significantly slower and the larvae pupated later than in control, but no significant difference in the final body mass was found between treatments. Consequently, there was no Cu effect on pupa mass. All insects that reached the pupal stage emerged. Copper affected time from hatching to adult: the higher the concentration the later the adults emerged. The fecundity dramatically decreased with increasing Cu concentration, from 502 to 42 eggs per female. Only at 1000 mg/kg the fecundity did not differ significantly from control. No effect of Cu was observed in sex ratio or survival of emerged adults. The experiment clearly shows that mortality-based bioassays may not detect toxic effects at the very beginning of their occurrence. Thus, they actually seriously affect key life-history traits.

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TU028
Regulation of internal copper concentration by the red flour beetle Tribolium castaneum
K. Stepień, Jagiellonian University / Institute of Environmental Sciences

In order to predict internal metal concentrations in animals under specific environmental concentrations (pollution levels), the relationship between exposure concentrations and values of toxicokinetic parameters must be known. Our previous studies in larvae of T. castaneum showed that the accumulation (kA) and elimination (kE) rate constants are usually constant only at certain conditions, such as specific metal concentration. For example, crickets exposed to metals in food regulated internal body concentration of Zn by changing simultaneously kA and kE whereas Cd was regulated almost exclusively through increasing kE. Whether other nutritional and xenobiotic metals are regulated in a similar manner, even if with different efficiency, it is not known. At high exposure concentrations, availability of the carriers by which metal ions are transported through membranes may become limited, and thus assimilation rates may decrease. Moreover, the higher the metal concentration in food the higher the damage to the gut and the lower the assimilation efficiency and/or the higher the elimination rate (mostly due to shedding damaged epithelial cells). Thus, the assimilation rates should decrease and elimination rates should increase with increasing metal concentrations in food. Indeed, our toxicokinetics study on the red flour beetle Tribolium castaneum (Coleoptera: Tenebrionidae) exposed to Cu at four different concentrations (500, 1000, 2000 and 4000 mg kg\(^{-1}\) dry flour) showed that with increasing Cu concentration in exposure medium, kE decreased from 0.0032 day\(^{-1}\) at 500 mg kg\(^{-1}\) to 0.0016 day\(^{-1}\) at 4000 mg kg\(^{-1}\). No differences between sexes in kinetics parameters were observed. Although a clear increase in body Cu concentration with increasing exposure was found, Cu was regulated by T. castaneum efficiently: an 8-fold increase in exposure concentration (from 500 to 4000 mg Cu kg\(^{-1}\)) resulted in only 1.7-fold increase of internal concentration. There was no effect of Cu exposure on respiratory metabolism of T. castaneum measured during toxicokinetics study. The project was financed by the National Science Centre project HARMONIA (No. 2012/06/M/NZ4/00137) and Jagiellonian University in Kraków (DS 758).

TU029
Soil Enzyme Activity Index vs. heavy metals Pollution Load Index. An ecotoxicological study in irrigated mixed farming in Morocco i.e. a common situation under semi-arid Mediterranean climate
J. Thoisy, F. Elsaa, INRA; J. Duplay, Strasbourg University; N. Rais, Fes University; V. Grondin, INRA; N. El Ghachtouli, A. Kouchou, Fes University; N. Cheviron, INRA

Many soils with relatively high pH and carbonate, and low organic matter contents under semi-arid climate conditions, often exhibit low metal mobility and increased metal accumulation at the soil’s surface (Lafuente et al., 2008). Thus, they may be considered as the most vulnerable soils (Batjes, 2000). Extracellular enzyme activities are good sensors for long-term soil metal anthropogenic contamination and recognized as more sensitive bioindicators than plants and animals (Hinojosa et al., 2004; K?l?/Kalyara, et al., 2004). In Europe, many studies on microbial disturbance by metals refer to Northern countries, compared to Mediterranean area (de Santiso-Martín et al., 2013). We focused on disruption study of biological parameters (impacts of exposure of microorganisms to heavy metal mixtures), by metal pollutants of the iron transition group (Cr, Ni, Cu, Zn) in calcareous agricultural soils (Saiss plain, North Morocco). We selected 5 metal concentration-plotted samples on identical soils: 1) maximum contamination along Oued Fez downtown Fez city; 2) at the confluence of Oued Fez and Oued Sebou; 3)–4) downstream the confluence; 5) uncontaminated reference sample (along Oued Sebou upstream the confluence). We focused on soil hydrolase activities because of their wide use in assessment of biochemical disruption by metals: phosphatase, arylsulfatase, urease, arylamidase, b-galactosidase and glucosidase. Enzyme Activity Index (EAI), by a similar computing of metal concentration factors and intensity factors of enzyme activities. PLI and AEI profiles showed strongly identical tendencies. The strong correlations between hydrolase activities suggested coexistence, if not an interdependency of key biological processes, such as nitrogen mineralization/transformation, cellulose degradation together with organic matter mineralization/transformation. This was currently not assessed to impacts of industrial effluents on water and soil in this area. The terrestrial ecotoxicity is assessed for each metal separately. It would be interesting to use these calculated indexes to characterize terrestrial ecotoxicity of mixtures by simpler Life Cycle Impact Assessment taking into account the local responses of soil and microorganisms. The efficiency of the EAI, an economic approach for predicting the toxicity of metal mixtures, needs further investigation on microbial mechanisms.

TU030
Diet quality influences chronic toxicity of copper to the freshwater snail Lymnaea stagnalis
P. Vincenz, o.h. berteloet, GhEnToxLab (Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; K.A. De Schampaelaere, Ghent University (UGent) / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology GhEnToxLab unit

Lymnaea stagnalis is a very widespread species in Europe and frequently inhabits systems impacted by urban and agricultural activities, which suggests that they might be relatively insensitive to chemicals, including metals. However, based on laboratory ecotoxicity data, L. stagnalis is amongst the most sensitive organisms to Cu, Pb and Ni. Thus L. stagnalis chronic toxicity data are an important determining factor in chronic Species Sensitivity Distributions (SSD) that are used to derive exposure standards for hazardous concentrations for 5% of the organisms (HC5). However most chronic toxicity studies with freshwater snails are 14-day to 28-day juvenile growth studies in which lettuce is used as a food source. Lettuce may be a sub-optimal food source for juvenile snails because early juvenile life stages might have an incompletely developed feeding apparatus making these juveniles insufficiently proficient to feed on lettuce. In this regard we tested the effect of food source (lettuce vs. fish flakes) on copper toxicity in a fully randomized 28-day juvenile growth test with L. stagnalis using an artificial water with semi-static renewal. Endpoints measured were: survival, shell length and dry blotted weight. Growth rates (µ) for all individuals were calculated as the slope of LN(length) in function of time. Dose response curves were generated for all endpoints and EC5 values were calculated. The food source plays an important role in size-related endpoints such as shell length, dry blotted weight and growth rate. Fish flakes fed individuals in control conditions had a >60% larger shell than lettuce fed individuals in control conditions after 28 days. For dry blotted weight a 10-fold difference in 28-day EC10 values was found, with an EC10 of 7 µg/L for lettuce fed snails and an EC10 of 78 µg/L for fish flakes fed snails. Also for survival, a 4-fold increase in EC10’s was found between lettuce and fish flakes fed individuals. Thus lettuce fed individuals were more sensitive for copper based on size related endpoints than fish flake fed individuals. However there was no difference in survival between snails fed with fish flakes and snails fed with lettuce exposed to copper. We hypothesize that the difference in sensitivity of L. stagnalis individuals to copper between the two food treatments is likely due to the high protein content and softness of the fish flakes making it a better food source for juvenile snails than lettuce.

TU031
Effects of arsenic compounds on freshwater microalgae from different phyla
under suboptimal conditions. G. Gímenez Papiol; N. Roig, Universitat Rovira i Virgili; J. Sierra, Universitat Rovira i Virgili / Soil Science Unit; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering

Algal growth inhibition assay is extensively used in aquatic ecotoxicology. The standardized protocol accepts a varied array of microalgal species, nevertheless, it requires optimal culturing conditions and specific growth stages for a correct parameterization of results. It is also time-consuming, since it lasts between 48 and 96h. Several biosensors based on microalgae have been developed, many of them relying on dead microalgae containing active enzymes. The living microalgae attached to a biosensor usually are in suboptimal conditions, compared to those provided in laboratory or in the growth inhibition assay, because they must be immobilized or maintained in order to be part of the sensor. Moreover, for commercial purposes, preservation for storage conditions must be also taken into account. The general objective of this work is to study the performance of microalgae maintained in suboptimal conditions when exposed to a known contaminant, in order to evaluate their suitability as the biological part of a commercial biosensor. To achieve it, Nannochloropsis oculata (Bacillariophyceae), Chlorella vulgaris (Chlorophyta), Rhodopesmus subcapitatus (Bacillariophyceae), and Synechococcus sp. (Cyanobacteria), purchased from the Sammlung von Algenkulturen (SAG,GoettingenUniversity), were chosen as representative of freshwater microalgae phyla and microalgal models. Arsenic compounds were chosen as representative of heavy metal contamination in Canadian rivers. The standard growth inhibition assay was compared to suboptimal conditions (stationary growth stage, precultivated in two sites of a Portuguese coastal lagoon (Aveiro lagoon); o) Largo do Lobo (LAR) located in an Hg contaminated confined area, and (ii) São Jacinto (SJ) closer to the lagoon inlet and selected as reference site. Winter and summer conditions were considered. Eye wall was analysed for total Hg (iHg) and methylmercury (MeHg) levels, as well as for antioxidant responses (CAT, SOD, GPx, GR, GST), peroxidative damage and acetylcholinesterase (AChE) levels. Inorganic mercury levels determined by ICP-MS and MeHg levels, iHg, iHg and MeHg in eye wall were higher at LAR than SJ in winter and summer, reflecting environmental spatial differences of water column and surface sediments. Moreover, fish caught at LAR in winter showed a significant decrease of CAT and SOD, in line with the occurrence of peroxidative damage. A different spatial pattern was recorded for those biological endpoints in summer, being characterized by the increment of GR and GPx at LAR, eventually preventing the occurrence of lipid peroxidation. The PCA analysis allowed discerning a spatial pattern was recorded for those biological endpoints in summer, being characterized by the increment of GR and GPx at LAR, eventually preventing the occurrence of lipid peroxidation. The PCA analysis allowed discerning a spatial pattern was recorded for those biological endpoints in summer, being characterized by the increment of GR and GPx at LAR, eventually preventing the occurrence of lipid peroxidation. The PCA analysis allowed discerning a spatial pattern was recorded for those biological endpoints in summer, being characterized by the increment of GR and GPx at LAR, eventually preventing the occurrence of lipid peroxidation. The PCA analysis allowed discerning a spatial pattern was recorded for those biological endpoints in summer, being characterized by the increment of GR and GPx at LAR, eventually preventing the occurrence of lipid peroxidation. The PCA analysis allowed discerning a spatial pattern was recorded for those biological endpoints in summer, being characterized by the increment of GR and GPx at LAR, eventually preventing the occurrence of lipid peroxidation. The PCA analysis allowed discerning a spatial pattern was recorded for those biological endpoints in summer, being characterized by the increment of GR and GPx at LAR, eventually preventing the occurrence of lipid peroxidation. The PCA analysis allowed discerning a spatial pattern was recorded for those biological endpoints in summer, being characterized by the increment of GR and GPx at LAR, eventually preventing the occurrence of lipid peroxidation. The PCA analysis allowed discerning a spatial pattern was recorded for those biological endpoints in summer, being characterized by the increment of GR and GPx at LAR, eventually preventing the occurrence of lipid peroxidation.
benchmarks are no different than water or sediment quality benchmarks, for which there is no expectation that a single water or sediment concentration affects all species equally. Thus, as is the case for water and sediment quality benchmarks, a reasonable expectation for screening level ecological risk assessment tissue benchmarks for metals that is they represent residues that, if not exceeded, are protective of a large proportion (i.e. 90 or 95%) of the aquatic species present in a community. The most detailed and species specific information is obtained from tissue residues associated with specific pollutants, such as lipids. The concentration of biologically active metals as opposed to whole body residues, are more appropriately performed during baseline ecological risk assessments, not during screening level risk assessments.

TU036
Effect of Ni, Zn and their mixtures on different morphological variables of duckweed
s. martinez, Universidad Nacional de Lujan; W.D. Di Marzio, Universidad Nacional de Lujan-CONICET; J. Alberdi, UNLU; S. Curieses, ma. saenz, Universidad Nacional de Lujan-CONICET

Lemna sp., commonly called duckweed, is a floating macrophyte commonly used in environmental risk assessment. Macrophytes are important in oxygen production, controlling water quality, sediment stabilization, providing habitat and shelter for aquatic life and wildlife and are primary energy sources for most aquatic ecosystems. Fresh water systems contaminated with heavy metals of thousands of industrial and natural chemical compounds is one of the major environmental and human health problems worldwide. In this work we evaluated the toxicity of two heavy metals (Zn and Ni) to duckweed. The aim of this study is to provide phytotoxicity data for the environmental hazard assessment process by comparing the effect on morphological and growth parameters of plants exposed to different concentration of metals. For this, monocultures of plants were exposed to different concentrations of Zn and Ni, in the form of ZnCl2 and NiSO4. Controls consisted in culture medium without metal addition. Plant were grown in an Erlenmeyer containing 250 ml of culture medium without metal for 7 days under a mean light intensity of 5000 lux. Different variables were measured: growth rates, frond and colony number, total frond area, fresh and dry weight and longitudinal and transverse axes average. Total area of fronds was calculated by an image analysis program (ImageJ). Growth rates, calculated by frond number, were affected by both metals. IC50 values derived from frond number were 1.51 and 123.89 mLg for Ni and Zn, respectively. IC50 values based on average fresh weight, were the lowest between all assessed variables, for both metals. All Ni concentrations evaluated differed significantly from controls when assessed by total area, frond number, colony number and fresh weight. While only the highest concentration showed significant difference from control in dry weight and longitudinal axis. No significant differences were observed with longitudinal axis average of fronds exposed to Ni showed no differences between treatments and controls. None Zn assay concentrations showed significant differences with controls on colony number and longitudinal and transverse axes average measurements. For dry weight and total area, only the two highest concentrations were different from controls. When frond number was evaluated, all Zn concentrations differed from control except the lowest. Fresh weight parameter was significantly affected at all assay Zn concentrations.

TU037
Mixture toxicity of copper and cadmium in the zebrafish (Danio rerio) K I. Condrey, Universiteit Antwerpen / Biology; R. Josserand, University of Antwerp; E. Goethals, Lebensmittel analysenwesen, Technologie & Management; K.A. De Schamphelaere, Ghent University (UGent) / Laboratory for Environmental Toxicology and Aquatic Ecology; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research Department of Biology

The uptake and toxicity of metals is well characterized for a number of aquatic species and this information is used to develop environmental quality standards. Most studies performed so far have focussed on single metal exposures, however in the environment exposure is often more complex including multi-metal exposures. In this study we have investigated the uptake and toxicity (mortality) of copper (Cu) and cadmium (Cd) in adult zebrafish (Danio rerio) on the basis of 10-day single copper and cadmium exposure concentrations. To avoid confounding variables the experiments were conducted according to a partial factorial design covering a wide range of exposure concentrations and different water types ranging from very soft to very hard. In the single metal exposure the zebrafish were generally much more sensitive to Cu than to Cd and the toxic effects showed a strong dependence on water chemistry. Exposure of the fish to binary mixtures of the metals resulted in a very strong influence in metal toxicity, which was much higher than expected on the basis of classical mixture toxicity models such as concentration addition, i.e. Cu and Cd interacted synergistically. The body concentrations of Cu and Cd in zebrafish also strongly depended on the exposure scenario but there was no evidence of a strong Cu-Cd interaction for uptake. As a result expression of metal toxicity in terms of internal metal loading only partially explained the observed toxic effects. These results demonstrate that metal interactions can have strong toxicological impact and that the observed mixture effects may imply synergistic interactions at the level of the homeostatic regulation and internal processing of the metals. More research is needed to identify if such interactions occur at low levels.

TU038
Subcellular partitioning of metals in the mealworm beetle (Tenebrio molitor) larvae Z.M. Swiatek, Institute of Environmental Sciences, Jagiellonian University / Institute of Environmental Sciences; A.J. Bednarska, Jagiellonian University / Institute of Environmental Sciences

Subcellular compartmentalization of the metals into organelles, heat-sensitive and heat-stable proteins (the first supernatant, S1 fraction), cellular debris (the second supernatant, S2 fraction) and metal-rich granules (G fraction) was evaluated after 7, 14 and 21 days of exposure using different centrifugation steps. In control animals the granular fraction represented 52% of the total accumulated Cd, and the percentage of Cd in fractions S1 and S2 was almost equal, ca. 24% each. Total Cd concentration (sum of all fractions) increased significantly with increasing exposure concentration. The percentage of Cd in the G fraction was similar (30-40%) in all Cd treatments. At the accumulation of Zn, the percentage of Cd in the S1 fraction, which represents metal trophically available to predators, was 1.7 times higher than at 600 mg/kg. With increasing Cd exposure from 100 to 600 mg/kg the percentage of the metal retained in S2 increased by a factor of 1.7. Cellular debris (S2) was the dominant fraction for Cd binding (46.5%) at the highest Cd concentration. Cadmium distribution in the mealworm larva will be compared with that of Zn for better understanding of sequestration processes of xenobiotic and nutritional metals and their possible transfer along trophic chains. The project was financed by the Foundation for Polish Science project HOMING PLUS (No HOMING PLUS/2013-8/1).

TU039
Searching for associations between mercury sediment loads, accumulated levels in gills and liver of the clam Venerupis philippinarum and oxidative stress profiles O. Araújo, DIVOA; A.M. Marques, CESAM e Departamento de Biologia; D. Pilió, F. Pereira, IPMA – Portuguese Institute for the Sea and Atmosphere; S. Guilleurme, Universidade de Aveiro / Health Sciences and Technology – KAUST / Red Sea Research Center; M. Pacheco, University of Aveiro / Dept of Biology; P. Pereira, Department of Biology and CESAM

One of the main challenges in Ecotoxicology is to establish comprehensive associations between environmental availability of trace elements and their accumulation in aquatic organisms, as well as between accumulated levels and biological effects. This is due to several confounding factors mainly related to the co-existence of numerous trace elements in environmental matrices, their bioavailability and the organisms’ physiological condition. The current work was designed to search relationships between those three lines of evidence (environmental quality – accumulated metals – biological effects) in a chronically contaminated estuary by mercury (Hg) (Tagus estuary, Portugal). In autumn 2013, a field campaign was performed at two sampling sites, one located in the most contaminated area of the estuary (Barreiro - BAR) and another in a low contaminated area (Alcóche – ALC) situated in the estuarine natural reserve. Surface sediments were collected at both sites, as well as the clam Venerupis philippinarum. Gills and digestive gland of the clams were analysed for total Hg levels and the oxidative stress profile (enzymatic antioxidants, GSH content and lipid peroxidation - LPO) was characterized. Surface sediments and interstitial water were also collected in both sites for Hg determinations. Environmental levels of Hg corroborated that Barreiro basin is a highly contaminated area. Despite that, accumulated levels of Hg both in gills and digestive gland of clams were significantly higher in specimens collected at the ALC site. The influence between environment Hg levels and those accumulated in clams’ tissues was previously reported in other estuaries, which can fit in the “Hg accumulation paradox”. An inhibition of CAT activity was observed in the gills at BAR compared with the ALC site, while SOD and GR activities increased. The enhancement of those antioxidant defenses probably prevented the occurrence of lipid oxidation in gills from BAR. Moreover, a significant increase of SOD activity and GSH content were recorded in digestive gland of clams from BAR. Such antioxidant protection couldn’t prevent the occurrence of LPO. Organ-specific patterns were found regarding oxidative stress being the digestive gland a vulnerable organ to peroxidative damage. The current puzzling data reinforce the need of an integrated approach to an accurate diagnosis within the environmental health assessment.

TU040
Determining detection limits for uptake of metals in mixtures from soils using PRSTM and DGT probes.

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control groups of fish were maintained in clean water during the whole experiment. Following this period, fish were transferred and kept in Hg levels of inorganic Hg in fish blood. Thus, fish. To address this issue, the present study aimed to assess the genotoxic effect of compounds to the aquatic ecosystems, there is an evident scarcity of studies linking or causation? Proceedings of the Royal Society of Medicine 58:295-300. Johnson I, Sorokin N, Atkinson C, Rule K, Hope S-J, Propositions for the observed effects as in the Environmental risk of chemical pollution in Mediterranean basins is of special concern due to high emissions faced with low dilution capacities. The aim of this study is to evaluate the risk of chemical pollution in Mediterranean rivers using periphyton responses as a model to assess chemical impact. The study also aims to compare classical periphyton indicators of water quality (e.g. diatoms diversity) with new biomarkers of toxic stress (e.g. chlorophyll-a fluorescence parameters and antioxidant enzymatic activities). Nineteen sites in four Iberian rivers (Llobregat, Ebro, Júcar and Guadalquivir) were sampled in autumns 2010 and 2011. A total of 116 organic compounds were detected in surface waters (60 pharmaceuticals and hormones compounds, 11 personal care products and industrial compounds, 36 pesticides and 9 perfluorinated compounds), and 6 metals in periphyton matrix. The potential risk of this cocktail of pollutants was estimated based on the “Toxic Units (TUs)” approach for algae. Results of TUs indicate that the Llobregat and Júcar rivers were those with the highest environmental risk for algae. TUs based on metals bioaccumulation in biofilm were two orders of magnitude higher than those based on organic compounds, being Zn and Cu the metals with the highest risk. Recent developments in the community composition allowed to identify sites with high environmental risk. Community changes in these sites presented an altered diatoms composition, where sensitive species to pollution were replaced by tolerant ones. Biomarkers, based on antioxidant enzymatic activities (catalase, glutathione reductase and superoxide dismutase) and chlorophyll-a fluorescence parameters (photosynthetic activity and non-photosynthetic quenching), tended to show a less consistent pattern. Overall, results indicate that in all the Mediterranean basins studied there are many sites with an environmental risk from chemical pollution, higher in exposed fish in relation to control, during both exposure and post-exposure periods, reaching its maximum value at E14. After 28 days of permanence in Hg-free water (PE28), Hg blood levels decreased to values similar with those found at E3, despite still significantly higher than the respective control. The frequency of ENA observed in exposed fish was significantly higher in relation to control and these levels were maintained during the entire experiment, revealing the genotoxicity of inorganic Hg. Overall, the prominent Hg levels in D. sargus blood along the experiment and at the post exposure period, resulted in non-recovery in what concerns the cytogenetic damage evaluated. These facts point out the environmental risk of the inorganic Hg to fish, underlying its genotoxic potential and long-lasting effect.

TU043 Predicting the chemical and biological effects of tertiary metal mixture (Ni, Cu, Cd) to aquatic plant, Lemma minor B.A. Hale, Y. Gopalamipillai, University of Guelph / School of Environmental Sciences

In nature, contamination of aquatic environments may be a mixture of metals and this is particularly true of natural waters contaminated by mining effluents since mineral deposits are commonly an association of multiple metals. For example, nickel mineral deposits mined from Sudbury (Ontario, Canada) are often associated with copper and cadmium. However, water quality guidelines (WQGs) for protection of aquatic life are not designed for multiple contaminants or multiple forms of the same contaminant that co-occur. Rather, WQGs are overwhelmingly based on dose-response studies of single contaminants. Resolving the inability to predict risk from metal mixtures in waters surrounding Canada's many current and legacy extractive mining sites is a high priority for Environment Canada, Health Canada, as well as base metal mining companies. Mixtures of forms of the same metal and mixtures of different metals may have toxicity that would not be predicted additively. Our study aimed to validate the 'concentration addition' approach to predicting the toxicity of a tertiary metal mixture (Ni, Cu, Cd) to Lemma minor (one of Environment Canada’s recommended test plant species for bio-monitoring of mining effluents) using a central composite design (CCD). The CCD is an efficient experimental approach that uses a rotatable incomplete factorial design to model a response surface, without requiring a complete three-level factorial, which is very resource intensive for conducting toxicity studies. Preliminary results show that toxicity of Ni, Cu, plus Cd is not concentration additive, likely due to competition amongst the metals for uptake into plant.

TU042 Linking inorganic mercury accumulation in Diplosopus saurus blood with the induction of erythrocytic nuclear abnormalities A.M. Marques, CESAM e Departamento de Biologia; P. Pereira, Department of Biology and CESAM; J. Raimundo, IPMA / DIVO/A; O. Araújo, S. Guilherme, Universidade de Aveiro / Department of Biology; F. Pousão, Setubal Institute for the Sea and Atmosphere; M.A. Santos, CESAM & Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal / Chemistry; M. Pacheco, University of Aveiro / Dept of Biology Despite the large knowledge and concern regarding the impact of mercury (Hg) compounds to the aquatic ecosystems, there is an evident scarcity of studies linking the natural environment due to fish mobility or contaminant input in parallel to fish. To address this issue, the present study aimed to assess the genotoxic effect of inorganic Hg in fish blood. Thus, Diplosopus saurus (=8) was exposed to realistic levels of inorganic Hg (2 μg L⁻¹) in water during 3 (E3) and 14 days (E14). Following this period, fish were transferred and kept in Hg-free water during 28 days (PE28), aiming to simulate the cessation of exposure that might happen in natural environment due to fish mobility or contaminant input in parallel. At each sampling moment, total Hg (Hg₀) was quantified in fish blood and the genotoxic potential assessed through the erythrocytic nuclear abnormalities (ENA) assay in mature peripheral erythrocytes. Levels of Hg₀ in blood were significantly

TU041 Differentiation between intrinsic toxicity and other effects - the question of causation R. Wees, Harlan Laboratories Ltd / Global Registration and Strategic consulting Iron salts exist dry in the oxidation stages +2 (ferrous salts) and +3 (ferric salts), but rapid hydration occurs in the case of ferrous, for addition oxidatively processes start in aerobic aqueous solutions. Such processes may cause death of test organisms by oxygen depletion, foulings effects and due to ingestion of flocculated matter. Nonetheless these substances were used in a number of studies for aquatic toxicity, which were available from the overview publications of Johnson et al. (2007) and Vymazal & Vymazal (2004). The HH and BL criteria for the in context of effect causation of pharmaceuticals, were adapted in order to differentiate between intrinsic effects and other reasons for the observed effects as in the regulatory context (e.g. REACH) only intrinsic toxicity matters. In result a scheme is presented, which is proposed for application in the evaluation process of metal salts tending to hydroxide formation and/or oxidation or generally formation of leave insoluble compounds. Hill AB (1964). The genotoxic metal concentrations in the soil. From this study, we will determine detection limits for PRS™ and DGT relative to total and bioaccessible soil metals. This information will be critical in determining if these tools may be applied to risk assessment of metals mixtures in soil.
and that the current use of periphyton biondicators and biomarkers allow to identify sites presenting high levels of pollution, however, new tools are required to evaluate “in situ” situations of moderate and low chemical pollution.

TU045 Metal contamination under high vigilance: The case of small streams in Northwestern Canada

L. Paris, INRS Centre Eau Terre Environnement; I. Lavoie, INRS - Eau, Terre et Environnement / INRS-ETE; S. Leguay, INRS-ETE; C. Fortin, University of Quebec / Centre Eau Terre Environnement

The fragile and pristine ecosystems of Northern Quebec (Canada) are under pressure from the ongoing and expected development of mining activities. Despite tight regulations, permanently ongoing environmental monitoring and information, metal contamination is likely to occur and aquatic ecosystems are particularly at risk. Monitoring metal contamination in streams is complex: trace metals in the water column show marked fluctuations in concentrations over time, bioavailable concentrations are difficult to estimate, and commonly measured parameters do not necessarily provide adequate information about the exposure and response of the biota. Combining chemistry measurements and biomonitoring is an appropriate approach for adequate water quality assessment. As primary producers, algae are particularly efficient for assessing the level of metal bioaccumulation and potential transfer to higher organisms. Studies have shown clear relationships between free metal concentrations ([M⁺]) in the water and intracellular metal concentrations in biofilms, suggesting that metal content in biofilm cells represents a robust indicator of contamination. This study aimed at (1) to test the consistency of this relationship for a wide range of species and to verify the efficiency of biovolumes (or cell size) in some species (e.g. Amphipleura pellucida var. dissipata and Nitzschia fonticola) also changed with arsenic exposure. A general trend in biovolume decrease was observed (Amphipleura pellucida, Nitzschia dissipata var. dissipata and Navicula reichardtiana var. reichardtiana), but Fragilaria species biovolume increased under arsenic exposure, due to greater cell and/or higher cell numbers in the arsenic treated. Measured biovolumes were compared with theoretical biovolume data for each species and were poorly correlated, suggesting that the real measurements are recommended to be used for a real impacts. In conclusion, both functional and structural algal parameters were affected by arsenic exposure. The strongest effect of arsenic on diatom community was the decrease in real cell biovolume, but arsenic also selected for species like Amphipleura pellucida (tolerant to metal exposure) and reduced diatom species richness. Considering how low is the arsenic concentration and exposure time in this experiment compared with reality, the results call into question the limits of arsenic concentration established to environmental quality, which are higher.

TU046 Periphytic diatoms responses to a short-term arsenic exposure

L. Barral, University of Girona / Environment; S. Morin, Iresta / UR EABX; H. Guasch, Institute of Aquatic Ecology

Periphytic diatoms are cosmopolitan aquatic organisms and are a major component of periphyton. They are also the basis of the trophic chain, thus they can respond quickly to environmental changes such as water metal contamination. Effects of metals on diatom community have been extensively studied. In field and laboratory experiments, underlying their high potential for metal contamination assessment. Arsenic pollution is a global problem. Water (surface and groundwater) is the main route of arsenic contamination. We conducted an experiment by combining ecological and ecotoxicological descriptors to investigate the effects of arsenic (130 μg/L) during 13 days) on functional and structural descriptors. Specifically, we analysed changes in photosynthesis-related genes and diatom community structure (species relative abundances) and species cell size. Arsenic exposure caused inhibition of periphytic algal growth and diatom community adaptation. We identified 52 diatom taxa of which Achnanthes minutissima was the most abundant species, representing almost the 77% of the total abundance of diatoms: 75% in control and 79% in arsenic exposure, showing a tolerance to arsenic. Moreover, arsenic reduced diatom species richness (p<0.051). Diatom average cell biovolume was clearly reduced by arsenic (p<0.003). Individual cell biovolume (or cell size) in some species (i.e. Nitzschia fonticola) also changed with arsenic exposure. A general trend in biovolume decrease was observed (Amphipleura pellucida, Nitzschia dissipata var. dissipata and Navicula reichardtiana var. reichardtiana), but Fragilaria species biovolume increased under arsenic exposure, due to greater cell and/or higher cell numbers in the arsenic treated. Measured biovolumes were compared with theoretical biovolume data for each species and were poorly correlated, suggesting that the real measurements are recommended to better assess real impacts. In conclusion, both functional and structural algal parameters were affected by arsenic exposure. The strongest effect of arsenic on diatom community was the decrease in real cell biovolume, but arsenic also selected for species like Achnanthes minutissima (tolerant to metal exposure) and reduced diatom species richness. Considering how low is the arsenic concentration and exposure time in this experiment compared with reality, the results call into question the limits of arsenic concentration established to environmental quality, which are higher.

TU047 LINKING NUTRIENTS, PHYTOPIGMENTS AND MICROFLORA IN RIVER BED SEDIMENTS: A CASE STUDY IN THE ANLLONS RIVER (SPAIN)

D.M. Prieto, University of Santiago de Compostela / Soil Chemistry; R. Devesa-Rey, University of Vigo; R. Paradelo, University of Vigo / Departamento de Biología Vegetal y Ciencia del Suelo Facultad de Ciencias Universidad de Vigo Campus de Ourense; M. Penalta, University of Santiago de Compostela; F. Díaz-Fierros Vigueira, University of Santiago de Compostela / Departamento de Edafología y Química Agrícola Facultad de Farmacia; M. Barral, University of Santiago de Compostela / Departamento de Edafología y Química Agrícola Facultad de Farmacia

In the Anllons River (NW Spain) several areas have been identified as affected by diffuse pollution due to agricultural runoff and industrial activities, as well as by past mining activities which originated elevated As concentrations in the bed sediments, downstream Au-As mineralized areas [1,2,3]. The Anllons riverbed sediments were analyzed to evaluate the presence of benthic epipsammic microflora and to identify the influence of nutrients and pollutants on the biofilm formation. Sediment samples were collected at four points along the river course. The study included the characterization of the pore waters: pH, electrical conductivity (EC) and total and soluble P. As, as well as the determination of the main chemical properties of the sediments: particle size, total and bioavailable P, total C, As and Nd, as well as toxicity, evaluated by TCLP extraction and Microtox® test. Biological properties were assessed by means of biochemical properties: phytotigoments, and dehydrogenase (DHA) and phosphatase (MPE) activity, and by means of a taxonomic study, requiring a specific sampling, to identify the main autochthonic species forming the biofilm. The main autotrophic taxa belonged to: Cyanophyta, Heterokontophyta, Euglenophyta and Chlorophyta. Long-term effects of toxicants that have exerted a selection pressure on the predominant class throughout the river bed sediments.Site 4, located at the river mouth, exhibited the highest values of nutrients, EC and biological properties (Chi a = 34.6 μg g⁻¹, DHA = 3075 mg TPF kg⁻¹ d⁻¹, MPE = 62.9 mg p-nitrofenol kg⁻¹ hora⁻¹), as well as total abundances (265,504 abundances cm⁻²) and species richness (17.8), and the lowest toxicity. Statistical analysis revealed significant, positive correlations (p<0.05) between chemical (except sand content and toxicity) and the biological properties of the sediments, evidencing the effect of the environmental conditions on the development of biofilm. [1] Devesa-Rey et al. 2008. Water Air Soil Poll 195 (1-4):189-199 [2] Devesa-Rey et al. 2010a. J Environ Manage 91 (12):2471-2477. [3] Devesa-Rey et al. 2010b. Environ Earth Sci 61 (7): 1401-1417.

TU048 Development of a dual radiolabel incorporation assay for simultaneous detection of toxicity to photosynthesis and bacterial protein synthesis in marine periphyton communities

M. Eriksson, Chalmers Technical University / Department of Shipping and Marie Technology; V. Fihlman, T. Backhaus, University of Gothenburg / Department of Biological and Environmental Sciences; H. Blanck, Goteborg University / Department of Biological and Environmental Sciences

Short-term toxicity assays are used to detect Pollution-Induced Community Tolerance (PICT) in microbial communities. In this context, they hence detect long-term effects of toxicants that have exerted a selection pressure on natural communities in the field or in experimental ecosystems. Various short-term metabolic endpoints have been used to detect PICT, but so far they have only studied effects on metabolic processes in a specific part of microbial communities, such as enzymatic activity in bacteria or photosynthesis in phototrophs. Here we present a methodology that simultaneously detects effects on bacterial protein synthesis and algal and bacterial photosynthesis by incorporation of 14C-labelled leucine and 3H-labelled bicarbonate, respectively. Since the decays of 14C and 3H have different energy spectra, liquid scintillation counting can simultaneously measure decays of these isotopes, and hence estimate both the amount of incorporated 14C and that of 3H in one sample. We tested incorporation of both 14C-labelled leucine and 14C-labelled thymidine, representing bacterial protein and DNA synthesis respectively, simultaneously to the incorporation of 3H-labelled bicarbonate into photosynthetic products. All three isotopes were linearly incorporated over the studied time of 8 hours, and neither 14C-labelled leucine nor 14C-labelled thymidine interfered with the counting of 3H-bicarbonate, and vice versa. Dark incubations revealed that leucine incorporation, but not thymidine incorporation, was affected. Incorporation of 3H-labelled bicarbonate seemed to be specific for protein synthesis in bacteria and photosynthesis in cyanobacteria and algae, respectively. During initial toxicity testing, incubation times of two, three and four hours were tested. The two endpoints of the assay responded differently to toxicants with specific mechanisms of action. For example, toxicity of a photosystem-II-inhibiting herbicide to photosynthesis was detected, but toxicity of a protein synthesis-inhibiting antibiotic was detected to bacterial protein synthesis, but not to photosynthesis. Hence, the dual assay could detect specific toxic effects on processes in the two organism groups, simultaneously. However, effects of more chronically acting compounds, such as the herbicide metazachlor and the antibacterial sulfanilamide, could not be detected over the studied incubation times.

TU049 Periphyton metaproteomics, from community ecotoxicology to environmental assessment in aquatic environment
As the general public since it is associated with ecological, societal and economic problems. Pioneering analytical methods have been applied to investigate this emerging contaminant. Still there is uncertainty as to the baseline values for the level of microplastic in marine environments, which is important for being able to assess trends. Furthermore we have only begun understanding its sources and sinks. To address these and other research questions robust, validated and comparable analytical methods are needed. In this project current methodologies were tested and modified for analyzing microplastic in sediment and invertebrate biota from different aquatic environments. Recovery in the modified method for particles in sediment increased by 18 % to 82%. For biota the optimized method gave an increase in recovery of 63% to a total of 97%. Raman spectroscopy showed promise for identifying microplastic from environmental samples, but certain obstacles were identified that will have to be considered for future investigations. The environmental measurements performed showed that microplastic was present in 85% of the biota samples investigated. It also indicated an accumulation of microplastic higher up in the foodchain and show a significantly higher amount in filter-feeders compared to other functional groups. In mussels the concentration was a thousand times higher than in surrounding water and sediment. These overall promising results with method developments are of high importance in developing monitoring strategies of microplastic. The method for biota analysis also provides the possibility to analyse whole-body residues and tissue concentrations of particles in organisms, but may also be applied to stomach microplastic content-analysis for particles as small as 1 μm. Variations between subsamples are however still high and fit-for-purpose experimental design and statistical methods are also needed to polystyrene, polyethylene, and polypropylene (PP) by FT-IR, but were not counted as microplastics using the microscope. And less than 1 mm sized fragment had a underestimated and fiber was significantly overestimated using the stereomicroscope both in the SML and beach samples. In the fragment, many plastic-like fibers such as sea grass and cotton were apparently miscounted as microplastics. The total abundance by FT-IR was higher than by microscope both in the SML and beach samples, but they were not significantly different because of the offset of the fragment and fiber in abundance. Depending on the number of samples and the microparticle size range of interest, the appropriate identification method should be determined; selecting a suitable identification method for microplastics is crucial for evaluating microplastic pollution. Keywords: microplastics, identification, stereomicroscope, FT-IR microscope

TU053 Mitigation of microplastics impact caused by textile washing processes. MERMAIDS project M. Escamilla, LEITAT / Sustainability Unit; R. Villalba, Leitat Technological Center / Sustainability Unit; L. Piñol, L. Rodriguez, Leitat Technological Center / Marine Division; R. Villalba, Leitat Technological Center / Biotechnology; M. Avella, CNR Institute for Polymers Composites and Biomaterials / ICTP; J. Lancharo, Polysitec S.L.; J. Dagevos, Plastic Soup Foundation / Programs European oceans are contaminated by marine litter, mainly by plastics. Microplastics are particularly worrying because water treatment plants do not take them into account in their management processes and they are deposited in waterways and sewage sludge. The European Marine Strategy Framework Directive (2008/56/EC) establishes a framework in order for Member States to contribute to create a Good Environmental Status (GES) in the marine environment by 2020 through the monitoring of different indicators, among them microplastics. Microplastic particles of synthetic clothes coming from laundry wastewater have been encountered in marine sediments, ecosystems and runoff and sewage waters. The “Accumulation of Microplastic on Shores: Sources and sinks” study states that “the source of the microplastic fibres in the sewage treatment plants is most likely to be from washing machine wastewater as the mixture of fibres...
found in synthetic textiles is similar to the mixture of microplastic fibres found in beaches at disposal sites and in the wastewater of sewage treatment plants.\(^2\)

On average, more than 1900 fibres of microplastics can be released by a synthetic garment during one wash\(^1\). Microplastics concentrate Persistent Organic Pollutants via partitioning and they can be ingested by marine biota entering in the food web\(^2\). The main objective of MERMADIS is to contribute to the mitigation of the impact caused by micro and/or nanoplastic particles resulting from laundry washes. A shift in the EU environmental policy partly due to the implementation of innovative technologies and additives for laundry processes and textile finishing treatments. A reduction of at least 70% of the total amount of microplastic fibres that is currently discharged in laundry wastewater is sought by means of the improvements achieved. Policy recommendations will be set in order to promote the widespread implementation of technologies which promote garments’ microplastics retention in washing processes contributing to reach a GES by 2020. With the same aim in view, good practices guidelines on microplastic retaining for plastic fibres manufacturers, textile industry, detergent manufacturers and households will be elaborated.\(^1\) [Science for Environmental policy.DG Env.new alerts issue 272. 9 Feb 2012 [2]Teuten,E. et al 2007.Potential for plastics to transport hydrophobic contaminants.Environmental Science and Technology41:7759-7764

TU054 Characterisation of Microplastics in Personal Care Products
K. Ronner, Institute for the Environment, Health and Societies Brunel University / Institute for the Environment; M.D. Scrimshaw, Brunel University / Institute for the Environment

Microplastics are synthetic contaminants which have a global distribution and are present in the aquatic environment. Studies on plastic contamination in the aquatic environment have focused largely on macroplastics which are greater than 5mm in size, but recent research is focusing on microplastics which are less than 5mm in size. In the past the plastic content of skin care products was partly due to the inclusion of synthetic microparticles intentionally used as cosmetic ingredients in personal care products. Today, the frequent use of facial scrubs or toothpaste which contain microplastics especially polyethylene, release unknown quantities of microplastics through waste treatment plants to the aquatic environment. Waste treatment plants which have fine and coarse screens do not remove these microplastics from waste waters effectively, before discharge to the aquatic environment. The lack of a standardised protocol for the characterisation of microplastics has hindered progress in determining the concentration and impact on the aquatic environment. Polyethylene microplastics in two brands of toothpaste and facial cleansers were extracted using a density separation method and viewed using binocular microscope equipped with QCapture Pro 5.1 imaging software, at 40X magnification. In conclusion, the microplastics analysed in all products were less than 1mm in diameter. Microplastics in toothpastes showed similar size range of 80 – 433.3 µm and 80 – 431.7 µm; and facial scrubs showed size range of 170 – 495 µm and 91.67 – 253.1 µm respectively. However, the analysis of variance carried out showed that there was a significant difference (p< 0.05) in the mean size of microplastics for the four personal care products (F13, 1506) = 494.93, p = 2.573E-227. In addition, final results showed that one of the facial scrubs had a total count of 969.6 microplastics per ml of product, while one of the toothpastes had a total count of 145.2 microplastics per ml of the product. These were the highest and lowest microplastics count per ml of the products respectively.

TU055 Microplastics in a biological wastewater treatment plant and the receiving freshwater environment in Flanders, Belgium
L. Van Cauwenbergh, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; W. Van Echelpoel, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology GhEntToxLab unit; K. De Gussem, G. De Gueldre, Aquafin NV; M.B. Vandegehuchte, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology GhEntToxLab unit

The vast majority of studies dealing with microplastic pollution focus on the marine environment as it is regarded as the ultimate sink for all littered plastic. Rivers are often considered as red continuous suppliers of this waste, and sewage treatment plants (STPs) as major sources of microplastics in the Thames river systems upstream of The Netherlands. Sediment samples from sixteen locations were taken using a Van Veen grab or spade and water samples were collected at all sites. Sediment samples were initially searched by eye, followed by flotation and overusing using ZnCl\(_2\) solution. Plastics collected from the sediments were subsequently identified by Raman spectroscopy. Initial observations indicate that coloured and mammade particles are obviously visible in sediments from sites with high population densities compared to few evident manmade particles in sediments from more rural areas. Further analysis will allow for correlation of the plastic types and abundance with population density and sewage inputs to understand the distribution of plastics in river systems.

TU056 Presence and Abundance of Microplastics in the Thames River Basin, UK
A. A. Horton, Centre for Ecology and Hydrology; E. Lahive, Centre for Ecology & Hydrology (NERC); C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology; D. Read, NERC, Centre for Ecology and Hydrology / Halls Section; D. Spurgeon, Centre for Ecology & Hydrology

The global increase in plastic production has led to growing concern about the environmental impacts of plastics and their degradation products. Microplastics have been extensively observed and studied in the marine environment but little is known about their presence and abundance in freshwater environments. Although rivers are recognised as a significant source of microplastics to the oceans, they are less considered in studies of the environmental presence of microplastics and there are no data reported to date on microplastics in UK rivers (or indeed any freshwater bodies). This study aimed to identify and quantify the abundance and types of plastics in the Thames Basin where population densities and sewage inputs are well described. Ten sampling sites on the River Thames and its tributaries were selected, ranging from densely populated, urban areas to sparsely populated, rural areas. Sites are all downstream of sewage treatment works (STWs) serving known populations, allowing correlation between population density with plastic types and abundances found. In addition samples were collected from sites at known distances downstream of STW outfalls, as well as the effluent itself, to try and establish the proportion of plastics directly entering from STWs and its fate and transport pathways. Before sediments and water samples were collected at all sites. Sediment samples were initially searched by eye, followed by flotation and overusing using ZnCl\(_2\) solution. Plastics collected from the sediments were subsequently identified by Raman spectroscopy. Initial observations indicate that coloured and mammade particles are obviously visible in sediments from sites with high population densities compared to few evident manmade particles in sediments from more rural areas. Further analysis will allow for correlation of the plastic types and abundance with population density and sewage inputs to understand the distribution of plastics in river systems.

TU057 Occurrence of microplastic in the river Rhine and Meuse delta

Microplastics are traced. Hypothesized to be transported and thought to affect organisms in several freshwater and marine systems nowadays. Little is known about the distribution of microplastic along river deltas. Here we report the first inventory of the occurrence of microplastics that includes fresh, estuarine and marine surface water locations of the main river delta in The Netherlands. Sediment samples from sixteen locations were taken using a Van Veen grab or spade and water samples were collected at five locations by filtering 10 m\(^3\) in triplicate. In a laminar flow cabinet, the samples were treated by density separation, organic matter degradation and filtration. Subsequent microscopy analysis indicates maximum concentrations of 0.06 particles / g dry weight sediment and 0.3 particles / m\(^3\) water, with no apparent spatial pattern present. These concentrations are in line with concentrations found for microplastics in fresh and marine water elsewhere in Europe. The relatively low concentrations may point to a high retention and burial of microplastic particles in river systems upstream of The Netherlands.

TU058 Microplastic sampling in the Mediterranean Sea
F. Menegatti, Department of Marine Biology and Spatial and Social Change; B.M. Sosthenes, K. Syrberg, Roskilde University / Department of Environmental Social and Spatial Change; H.B. Pedersen, C. Sick, Plastic Change; M. Eriksen, 5 Gyres Institute; Y. Shashoua, The National Museum of Denmark; F.R. Khan, Roskilde University / ENSPAC

The extent of microplastic pollution in the Southwestern Mediterranean Sea is not yet known, although on Northern part has been previously studied. Plastic samples were collected at 7 transects during a 10 day expedition from Sicily (Italy) to Malaga (Spain) in September 2014. A 330 µM mesh manta trawl was used for surface water sampling. Physical and chemical characterization of plastic particles was performed with regard to size (1<5 and >5mm), shape (fragment, line, thin
film, foam and pellets), color (transparent, white, black and colored), density and chemical composition according to 5 Gyres protocol. Microplastics (< 5mm) were more abundant than macroplastics (>5mm) across all sites. Abundance decreased with increasing distance from the coast of Palermo to the open sea, with the highest abundance (2071 particles per km²) observed at the Sicilian coast. Fragments were the dominant shape of plastics with 86% and 75% of < 5mm and >5mm sizes, respectively. Transparent plastics comprised the majority of total plastic particles obtained with 65% and 56% of < 5mm and >5mm sizes, respectively. Despite their small number, plastics of >5mm weighed more than particles < 1mm. This is the first study to assess plastic pollution levels in SW Mediterranean Sea.

TU069 Microplastics in marine and freshwater environments of northern Scotland
N.A. James, Environmental Research Institute / Environmental Research Institute; B. Gordon, K. Boyd, University of the Highlands and Islands / Environmental Research Institute

As plastics have become more widely used, their presence in the environment has increased. Microplastics, small pieces of plastic measuring less than 5 mm in two of their dimensions, may have detrimental effects on the environment and its inhabitants. Sediment samples were collected from five marine beaches and two freshwater lochs in Caithness in the far north of Scotland. Microplastics were separated from sediments on the basis of density and enumerated visually using microscopy. Microplastic particles were found in all samples. The samples ranged from 0.07 to 37.09 pieces of plastic per 100 g. The greatest number of microplastic pieces (400,000 per 1m²) were found at Scrabster Beach, whilst the fewest (166.67 pieces per m²) were at Loch Calder. Four of the five saltwater samples and one of the two freshwater samples had significantly more plastic particles than in control samples. A wide range of different coloured plastics were found with clear being the most common, and predominantly rope fragments or fibres. In order to determine if such materials could be a vector for polycyclic aromatic hydrocarbons (PAHs) samples were collected from five beaches and analysed for the presence of phenanthrene and anthracene using high performance liquid chromatography with fluorescence detection. Extracts contained PAHs in concentrations over 200 times that of the control samples. The presence of microplastics, combined with their ability to adsorb PAHs in the environment highlights the importance of understanding their source and fate in remote areas such as Caithness, Scotland.

C. Schoenlau, Centre for Marine and Environmental Research / MTM research center Department of Science and Technology; T.M. Karlsson, Orebro University / MTM; P. Geladi, Swedish University of Agricultural Sciences / Unit of Biomass Technology and Chemistry; H. Grahn, Corpus Data Mining; M. Engwall, Orebro University / MTM research center Department of Science and Technology; B. van Bavel, MTM Orebro University; A. Kârmann, Orebro University / SCHOOL OF SCIENCE

Microplastics are defined as fibrous or particulate pieces of plastic smaller than 5mm and are commonly found in seawater, intertidal sediments, and other coastal environments. These plastics can enter the marine environment from the breakdown of macroplastics discarded at sea or on land as well as from consumer products containing microbeads. Microplastics need to be monitored in order to evaluate the effectiveness of the Scottish Government’s initiatives to reduce plastic debris in the environment. The aim of the present study, therefore, was to investigate the presence of microplastics at the south coast of Scotland, and particularly around the Shetland Islands. A total of 30 samples were collected from five beaches in Shetland and the Shetland Islands, with samples collected from coastal sites and offshore sites. The results showed that the majority of microplastics found were fibrous, mainly polypropylene, with a range of different colours and sizes. The most common type of microplastic found was fibrous, mainly polypropylene, with a range of different colours and sizes. The most common type of microplastic found was fibrous, mainly polypropylene, with a range of different colours and sizes. The most common type of microplastic found was fibrous, mainly polypropylene, with a range of different colours and sizes. The most common type of microplastic found was fibrous, mainly polypropylene, with a range of different colours and sizes.
investigated, but little is yet known about the abundance and types present on the Scottish coast. Preliminary (unpublished) results from Scotland show that microplastics can be found both among sediments and in the lumen of fish digestive tracts, especially near urban areas. Our goal was to establish a standardised procedure for extracting and quantifying the presence of MPs in marine mussels, and to apply this procedure to inter and subtidal species from the East and West coasts of Scotland. Collecting marine mussels in the Scottish species was Modiolus modiolus. Methods for extraction and quantification of MPs from marine organisms have not been established, and some traditional methods (e.g. use of strong acids or bases) for extracting substances from tissues are likely to damage MPs. We have compared previously reported methods and developed a new method based on enzymatic digestion that is relatively easy, reproducible, and provides good recovery rate of MPs from spiked tissue samples. Mussel collected from various field sites on the East and West coast of Scotland are being processed according to this procedure and we anticipate providing information on the level of MPs contamination in mussels according their site of collection and habitat. A field campaign is underway that involves transplantation of M. edulis into purpose built cages and use of passive water samplers to quantify MPs presence and abundance at specific sites on the Scottish coast.

TU064
Microplastic in a macro filter feeder: humpback whale Megaptera novaeangliae
Marine filter feeders are suspected to be exposed to microplastic because of their selection of small particles as food source. Bivalve, worm but also whale species feed on means of filtering, thereby harvesting small particles from the water volumes. Macroplastic, as well as phthalates possibly serving as indicators of microplastic, were found in whales before. This study is the first to show the presence of microplastic in the intestines of a humpback whale (Megaptera novaeangliae), which stranded in the Netherlands in 2012. After cutting away the left sided blubber and ribcage part of the stomach and intestines were removed. These were dissected, contents were rinsed sequentially over 1 mm and 0.5 mm sieves, dissolved in 10% potassium hydroxide and washed in double bags (mesh 120 μm and 300 μm) in a washing machine. From the remaining dried material, possible synthetic polymer particles were selected based on density and appearance and subjected to Fourier transform infrared (FTIR) analyses. The obtained FTIR spectra were compared with reference spectra by linear regression in ‘R’ statistical software. Particles for which the comparison with reference spectra had a quality index (R’) of >0.7 were identified as synthetic polymers. Several polymer types were found in the processed stomach and intestine content, in varying particle shapes: threads, sheets and fragments. The diversity in polymer types and particle shapes found, can be interpreted as a representation of the varying characteristics of marine plastic and the unselective way of ingestion by Megaptera novaeangliae.

TU065
Feedbacks between bioturbation and microplastic at the sediment-water interface
Dynamic processes at the sediment-water interface are crucial for either burial or resuspension of microplastics in aquatic systems. Biofouling and subsequent settling are thought to remove microplastics from the water phase, to enhance sediment concentrations and therewith to affect benthic organisms. It is unknown whether bioturbation affects the role of sediments as a sink for microplastics. During a 28 d exposure assay with 10–180 μm polyethylene, microplastic concentrations in the sediment were monitored in systems with and without the bioturbating marine lugworm Arenicola marina. Additionally, survival, feeding activity, body weight, and microplastic in faeces, gut content and tissue of A. marina were studied. Microplastic appeared to negatively affect the feeding activity of A. marina, which is in line with earlier reports. Microscopy analyses showed occurrence of microplastic in the gut content, while no indication of transport into tissue was found. After exposure, A. marina appeared to enhance the concentration of microplastic in the overlying water compartment. This study therefore shows that besides effects of microplastics on organisms, organisms can also affect microplastic distribution in surrounding compartments, implying a direct feedback between microplastic fate and effects. Using a simple sedimentation-resuspension model, the biodifussion coefficients were derived from the experimental data. Bioturbers can be hypothesized to affect the relative importance of sediments being a sink and the temporal bioavailability of microplastics.

TU066
Nanoplastic in the Aquatic Environment
A.A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group; E. Besseling, Wageningen University & Research Centre / Aquatic Ecology and Water Quality Management Group; W. Shim, Korea Institute of Ocean Science and Technology / Oil and POPs research group
An increasing body of literature reports on the abundance and effects of plastic debris, with an increasing focus on microplastic particles smaller than 5 mm. It has often been suggested that particles in the < 100 nm range as defined by nanomaterials, may be emitted to or forced in the aquatic environment. Nanoplastics is probably the least known area of marine litter but potentially also the most hazardous. This poster provides a review on sources, effects and hazards of nanoplastics. Detection methods are in an early stage of development and to date no nanoplastics have actually been detected in natural aquatic systems. Various sources of nanoplastics have been hypothesized such as release of non-nanomaterials, nanofragmentation of larger particles. Nanoplastics are found in water samples and in the overlying water compartment. This study therefore shows that besides effects on aquatic organisms, effects towards a fundamental and systematic understanding of the relevant sorption and bioavailability of大楼是微塑料 is of crucial importance and will significantly contribute to the hazard of plastics. Because of the presence of such co-contaminants, effect studies with nanoplastics pose some specific practical challenges. We conclude that hazards of nanoplastics are plausible yet unclear, which calls for a thorough evaluation of nanomaterial sources, fate and effects.

TU067
Sorption of polar and non-polar organic compounds by microplastics
T. Hüffer, T. Hofmann, University of Vienna / Department of Environmental Geosciences
Over recent years, the presence of plastic debris has become an issue of public concern as well as among scientist and regulators[1]. While so far the majority of studies have investigated the occurrence of microplastics (MP) in marine environments, there have few but growing number of reports on the occurrence of plastic debris in non-marine aquatic ecosystems. The input of MP presents a potential hazard to environmental ecosystems resulting from entanglement or ingestion of these particles by organisms[2]. There has also been a growing concern about an “indirect” effect, which results from plastic particle acting as a contaminant carrier[3]. To this end, sorption of organic compounds by MP is an important processes as it may affect the compound distribution in sediments and aqueous phases. For example, strong sorption of organic compounds by microplastics, even at comparable low sorbent concentrations, could consequently reduce the decrease of these compounds in sediments. Thus, a detailed knowledge of sorption properties of microplastics is of crucial importance and will significantly contribute to the discussion on potential relevance of the presence of microplastics in the environment. To date, research on sorption by MP mainly focuses on the investigation of individual sorption mechanisms by a rather limited number of organic probe sorbates and extensive solid phase characterization data of most commonly found MP. The results are expected to contribute to a better understanding of the molecular interactions to occur in the environment. [1] Cole et al. (2011) Mar.Pollut.Bull., 62, 2588-2597. [2] Wright et al. (2013) Environ.Pollut., 178, 483-492. [3] Rillig (2012) Environ.Sci.Technol., 46, 6453-6454. [4] Guo et al. (2012) Environ.Sci. Technol., 46, 7252-7259.

TU068
GC-MS screening of additive chemicals in plastic products from beaches and riverine deposits
M. Rani, Korea Institute of Ocean Science and Technology; S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; M. Jang, Korea Institute of Ocean Science and Technology / oil and POPs research group; OPRG; G. Han, Oil and POPs research group; Y. Song, Korea Institute of Ocean Science and Technology; W. Shim, Korea Institute of Ocean Science and Technology / Oil and POPs research group
Grav Street, Kansas City, Missouri, USA, Plastic additives are utilized in various applications because of their versatile. Due to their formulation and/or processing, these synthetic polymers contain additives and impurities which may leach out under conditions of use and accumulate in environment. The threat of plastics pollution to the marine environment has been ignored for a long time, and its seriousness has been only recently recognized. Therefore, to check the role of plastic debris in spreading these chemicals as contaminants in marine environment, a number of marine debris were collected from coastal beach and sorted to food (bowl, straw, cider and water bottles, packaging films of cookies, tissue box etc.), aquaculture (bouys, buoy rope, ropes, eel fish trap, fish trapper float, fish trapper disassembler) and general uses

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Internal diffusion in the plastic phase is a key process determining bioavailability of POPs sorbed to MPs.

TU071

Size-segregated HOCs contents in field-collected microplastics

I. Kim, Incheon National University / Department of Biology; S. Noh, Incheon National University / Department of Marine Science; S. Kim, Department of Marine Science, College of Natural Sciences, Incheon National University / Department of Marine Science

Microplastic contamination is an emerging issue in conservation of marine ecosystem. Expanded polystyrene (EPS), one of plastic polymer, is widely used in marine environment as aquaculture buoy and packing materials. A number of used EPS is lost and transported via current and wind to other region. Unlike other plastic debris, these transported EPS is easily fragmented and is segregated to particles with various sizes from nanometers to micrometers by weathering on beach where they reached. EPS was known to be a major constituent (on basis of counted number) of microplastics as well as debris found on high-stranded line of coastal sand beach. According to a recent study (abstract No. 301, 24th EU SETAC), considerable amounts of hexabromocyclododecane (HBCD) of several mg/g was detected from EPS buoy. This flame-retardant chemical newly listed-up in the Stockholm Convention can be easily released to environments (air or seawater) when EPS is fragmented because HBCD is not chemically bond to EPS but an additive. This indicates the possibility of EPS as a strong source of HBCD in marine environments. In this study, we collected EPS particles at each five stations of several sand beaches and sorted EPS with size as follows: particles over 1 mm and thereafter measured HBCD contents in each size group of EPS particles. From this study, we expect to be able to determine HBCD release rate by weathering (i.e., fragmentation) under the field condition.

TU072

Comparison of particle size on POP sorption to microplastics

E. Troia, University of Amsterdam / IBED; R. Helmus, University of Amsterdam; J. Westerveld, J. Parsons, University of Amsterdam / IBED

The distribution coefficients of polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs) between salt water and different sizes of polystyrene (PS), polypropylene (PP) or polyethylene (PE) were determined. The polymer sizes ranged from mm to μm and mm-sized particles were used for desorption study. To model for infinite sink, sufficiently high volume of seawater was used to provide a constant concentration of the poorly water soluble compounds in an equilibrium sorption experiments of 30 days. Phenanthrene adsorption was estimated to be almost one order of magnitude stronger to mm-sized PS compared to that for μm-sized PS. Adsorption of pyrene was 0.8 orders of magnitude stronger to μm-sized PP compared to mm-sized PP. In contrast, no significant differences were seen for PAH sorption to the three sizes of LD-PE polymer. Overall, an increasing trend was demonstrated for the adsorption of several hydrophobic contaminants to smaller plastic particles with increasing specific surface area. At PAH concentrations comparable to those in oceans a 6 fold concentration increase was estimated for PP particles that were 10 times smaller in diameter. Although the results are general and can be expected as sink and transport vector for hydrophobic contaminants, they do indicate that further studies, preferably with higher log Kow compounds, and including more nano-sized plastics are needed.

TU073

Distribution of polybrominated diphenyl ethers (PBDEs) in microplastics in coastal waters and open ocean: application of International Pellet Watch Project

J. Parsons, Department of Marine Science College of Natural Sciences Incheon National University; S. Kim, Department of Marine Science, College of Natural Sciences, Incheon National University / Department of Marine Science

Seawater is a medium for marine organisms directly exposed to pollutants. However, it is a great challenge to monitor extensively in time and space hydrophobic organic chemicals (HOCs) in seawater owing to difficulties in detection of trivial levels and variation of concentration via currents. Hydrophobic organic chemicals (HOCs) such as persistent organic pollutants sorbs to the organic matter of solid matrix in the marine environment. Thus, HOCs in sediments are a major constituent of the organic matrices and is the source of HBCD in marine environments. In this study, we collected EPS size, weathering extent, and source polymer, HOCs concentration desorbed on pellets will be compared with those in ocean and bed sediment collected the same beach. The result is expected to answer to the question of “is pellet-watch still applicable to HOC monitoring in local-scale marine environment?”.
concentration of lower brominated congeners was observed in pellets from Belize, though the PCB concentration was in background level. This is probably due to current inputs of PBDEs occurring in industrially developing country. Higher brominated congeners, mainly BDE209, was sporadically detected in pellets from USA and Japan. These suggest that BDE209 is compounded into plastic resin pellets intentionally or unintentionally (i.e., transfer from former products through recycling). Additives are desorbed from plastics during floating and fragmenting in the surrounding seawater. Older plastics (e.g. PE pellets from Tyko Bay and sorted into two size fractions (315 µm – 1000 µm and 1000 µm to 5000 µm) and analyzed for PBDEs and PCBs. Lower brominated congeners as well as PCBs were detected in both fractions of plastics. Both are thought to be derived from sorption from surrounding seawater. Higher brominated congeners were detected even in 315 µm – 1000 µm as well as 1000 µm to 5000 µm fractions. This indicates that BDE209 is not completely leached out and is still contained in µm-size plastics in coastal waters. We are analyzing PBDEs in microplastics (315 µm – 1000 µm and 1000 µm to 5000 µm fractions) in open ocean and the results will be presented on the poster.

TU074 Transfer and accumulation of organic pollutants in Atlantic salmon: Effect of incorporation of plastic particles in feed

M. Kotterman, IMARES / Fish; B. Larsen, University of Mississippi / Pharmacology; J. Sloth, DTU Technical University of Denmark / Food; K. Granby, DTU Technical University of Denmark

One of the objectives of the EU project ECSafeSEAFOOD is to assess food safety issues related to priority contaminants present in seafood as a result of environmental contamination, including those originating from harmful algal blooms and those associated with marine litter. In natural waters as well as in aquaculture carryover of contaminants from feed, suspended matter and water to fish can take place. In order to prioritize how to mitigate the levels of contaminants in the fish for human consumption the carryover should be estimated and modelled. The effect of plastic particles and different relevant priority contaminants from feed to fish as well as depuration of the fish is studied in an experiment with salmon, as case study for fish of high European dietary consumption. Control feed with low contaminant levels and feed spiked with contaminants at levels relevant for European waters are fed to the fish. To assess the effect of marine litter, ie plastic, on the level and type of contaminant uptake, the feeding experiment is also performed with inclusion of non-contaminated polyethylene microplastics (2% w/w, LDPE 125-250 µm) in both types of feed. The fish are kept in 600 L cages with a maximum of 100 kg fish each (full size) with saltwater and a flow-through system at DTU aqua Denmark. The exposure (feeding) period is 12 weeks, followed by an eight week depuration period. Samples (filets) will be taken every two weeks during exposure and two weeks during depuration. The contaminants being studied are brominated flame retardants, fluorinated compounds, PCBs and methyl mercury. In this poster we will show the preliminary results concerning PCBs as well as PCBs; uptake from the feed in the presence or absence of plastic particles. Acknowledgement - The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7-2007-2013) under the ECSafeSEAFOOD project (grant agreement nº 311820).

TU075 Modeling the relationship between POPs in ingested plastic debris and in tissue of Norwegian Northern Fulmars

D. Herzerk, NINA Research Institute for Air Research / MILK; B. Koelmans, Wageningen University; T. Anker Nielsen, M. Langset, NINA

Plastic has become a major part of marine litter and may threaten marine ecosystems seriously... Marine plastic can also act as a vector for transfer of hydrophobic chemicals polluting the oceans, potentially increasing the hazards of plastic to marine life. Recently, plastic-inclusive bioaccumulation models have been shown to be helpful tools to mechanistically understand transfer to marine organisms. In Europe, Northern fulmars have been used for quite some time to monitor plastic ingestion by seabirds, due to their longevity and spending almost their whole life off shore. Few data exist for Northern fulmars from Norwegian waters, but the load of plastic particles in fulmars beached in SW Norway is much higher and according to OSPAR (e.g. van Franeker et al. 2011). In 2012 and 2013, 84 fulmars were unintentionally caught as by-catch on long-lines off the coast of Northern Norway. Liver and muscle samples were collected together with plastic particles found in the stomach of each individual. Plastic particles (particles as well as fibres) were found in 82% of the birds stomachs, ranging from 1 to 127 pieces (0.002 - 0.725 g) per individual. Plastic particles were analysed for persistent organic pollutants in selected individuals (n=30). Birds with a high, medium and no load of ingested plastic were selected for comparison. The correlations (r2) of POP concentrations between liver and plastic samples originate from the same individual, ranged between 0.46 (PCB153 and 0.88 (pp'-DDE)). No correlation was found for PBDEs. Muscle samples of birds with ingested plastic did show no elevated levels compared to muscles of birds which did not ingest plastics. We implemented a novel plastic-inclusive bioaccumulation model for the fulmars. The model used the gradient between ingested plastics and bird lipids to calculate the driving force for transfer, used a gut clearance of ~70% of the ingested plastic per day and was applied to mechanistically explain the observed bioaccumulation data. When estimating the contribution of POP intake caused by the ingested plastic, a median contribution of 0.4, 0.3 and 7.0 ng/g ingested plastic of sumPCB, sumPBDE and sumDDTs can be found.

TU076 Adsorption of polycyclic aromatic hydrocarbons and AhR-mediated agonistic activity of lipophilic extracts of different plastic types after deployment in the San Diego Bay, California

M.M. Lam, RUTH University Aachen / MTM research center Department of Science and Technology; M. Larsson, Orebro University / MTM research center Department of Science and Technology; C.M. Rochman, UC Davis / Department of Ecology; G. He, University of California; M.S. Denison, University of California / Department of Environmental Science

OSPAR (e.g. van Franeker et al. 2011). In 2012 and 2013, 84 fulmars were monitored plastic ingestion by seabirds, due to their longevity and spending almost their whole life off shore. Few data exist for Northern fulmars from Norwegian waters, but the load of plastic particles in fulmars beached in SW Norway is much higher and according to OSPAR. Higher brominated congeners were detected even in 315 µm – 1000 µm as well as 1000 µm to 5000 µm fractions. This indicates that BDE209 is not completely leached out and is still contained in µm-size plastics in coastal waters. We are analyzing PBDEs in microplastics (315 µm – 1000 µm and 1000 µm to 5000 µm fractions) in open ocean and the results will be presented on the poster.

TU077 Bioavailability of microplastics and associated toxic substances for aquatic organisms - a food web experiment with Artemia nauplii and zebrafish (Danio rerio)

A. Batel, COS University of Heidelberg / Aquatic Ecology and Toxicology; M. Scherer, University of Heidelberg; T. Braunbeck, University of Heidelberg / Centre for Aquatic Ecology and Toxicology Studies

In this experiment, the biotransfer of microplastics along a simulated two-organism food chain was analyzed as well as the effect of potentially bound toxic substances on microplastic particles by ingestion using the EROD activity test. Microplastics are of major focus in the recent detection of new potential hazards for the aquatic environment. Many researchers showed that microplastics are ingested by a great variety of different organisms and aquatic animals and that all researchers share has not been sufficiently analyzed: To which extend are microplastics transferred via the food chain and do toxic substance attached to microplastics become available for organisms after ingestion? The two-organism food chain in this experiment consisted of freshly hatched Artemia nauplii and zebrafish (Danio rerio) under laboratory conditions. We first analyzed the ingestion of fluorescently labelled microplastics and associated toxic substances in the aquatic environment can be traced.
cause while flowing their way to the oceans. However, little is still known about their fate and impact on the freshwater community. Microplastics are particles of < 5 mm in diameter that can easily be ingested by a wide range of aquatic organisms, especially by filter feeders. Among them, bivalves are one of the most efficient filter feeding groups spread worldwide, which feed on all kind of suspended particles such as diatoms, algal cells, bacteria, small zooplankton and detritus. It is expected that suspended microparticles will likely be filtered by bivalves as well. Here, we present a study that conducted under different environmental conditions, aiming to assess the filtration and excretion of microplastic over time by adult duck mussels (Anodonta anatina). We used green fluorescence polyethylene (PE) microspheres ranging from 10 to 90 µm in diameter. A total of four treatments with different PE concentrations (0.01, 0.1, 1 and 10 mg/L) and the control (without PE) were performed and replicated in 60L-aquaria filled with 20L of copper-free tap water. Three mussels were placed in each aquarium and maintained under same light, temperature and aeration conditions in a climate room. Mussels were fed daily on a mixture of instant algae during the 5 day exposure assay. Every 24 hours water and excrated material (faeces and pseudofaeces) were collected. Valve opening and movement were also monitored daily. All the samples were analysed for microplastics by UV flow cytometry. Particle size was also analysed by flow cytometry and contrasted with measurements of particle diameter under the microscope. Observations on valve opening and excreted material showed that mussels were actively filtering in all treatments including the control. No treatment effect was observed in valve opening. Concentrations of microplastics were in all cases higher in the excrated material than in the water.

TU079
Long-term exposure of weathered microplastics from toothpaste (Mytilus galloprovincialis) - a case study
I. Nertel, Ecotoxicology and Risk Assessment; M. Blazquez, Marine Renewable Resources; P. Thomas, CEITIRA SAS / Ecotoxicology and Risk Assessment
Keywords: Microplastics, toothpaste, weathered, long-term exposure, bivalves
Microplastics are small plastic particles or fibers ubiquitously spread in the marine environment. This is threatening wildlife since several species are known to ingest microplastics. One source of microplastics released into the ocean is primary microplastics used in personal care products. These typically are released in treated wastewater, with an estimated that 2-4 mg of microplastics/person/day is introduced into the environment. A recent study looking at the toxicity of weathered microplastics to marine organisms is crucial for understanding the interaction between microplastics and organisms on a marine species.

TU080
Effects of microplastics on the toxicity of cephalixin to the common goby (Pomatoschistus microps) and modulation by temperature: predatory performance and biomarkers
E. Fonte, ICBAS CIMAR University of Porto; P. Ferreira, ECT Ekotoxicologie GmbH; I. Guilherme, CIMAR University of Porto
The contamination of estuaries by microplastics (MP) and pharmaceuticals and the effects of temperature on the toxicity of these substances raise a high concern in relation to their potential adverse effects on environmental and human health. More knowledge is needed to improve the basis for the risk assessment of these stressors. Thus, the effects of MP on the toxicity of cephalixin (CEP), a widely and intensively used antibiotic, to the common goby (Pomatoschistus microps), a key-stone species in several European ecosystems, were investigated at 20°C and 25°C. Short-term (96h) laboratory bioassays with early P. microps juveniles exposed to CEP alone and in combination with MP were carried out at both temperatures. Post-exposure predatory performance (PP), the activity of the enzymes acetylcholinesterase (AChE) and glutathione S-transferases (GST), and lipid and protein concentrations (lipid and protein) as effect indicators. At 20°C, exposure to cephalixin alone (low ppm range) significantly decreased the PP and increased GST activity; under simultaneous exposure to cephalixin and MP, the effects on PP and GST increased and AChE was significantly induced. The 5ºC of temperature rise under simultaneous exposure to cephalexin and MP, the effects on PP and GST are exploited on a larger scale and in a more environmentally friendly way.

Wildlife ecotoxicology from molecular to population effects (P)

TU081
Temporal variation of Water vole exposure to bromadiolone following the recolonization of a treated plot
M. Sage, Wildlife Environment Expertise; M. Coeurdassier, University of Franche-Comté / UMR ChronoEnvironment; I. Fouré, Vetagro-sup, campus vétérinaire / Toxicology; R. Defaut, S. Bagnoù, FREDON Franche-Comté; P. Giraudoux, Université de Franche-Comté / Laboratoire Chrono-environnement
The anticoagulant rodenticide bromadiolone is widely used as plant protection product to control Water vole outbreaks in European grasslands. Following a treatment, vole population declines rapidly and reaches very low density 3 weeks after bait applications. Then, the plot could be recolonized by voles coming from surrounding plots. When buried in artificial galleries, the persistence of bromadiolone is shown to be short (DT50 ~ 7-10 days). However, baits may be stored in cavities by voles, which increase dramatically the persistence of bromadiolone in the environment (DT50 from 25 to 43 days, Sage et al. 2007, Environ. Pollut. 147). In this study, we aim to document the exposure to bromadiolone of Water voles recolonizing a plot several months after a treatment. To simulate the recolonization, some water voles were trapped alive in non-treated groups and then reintroduced. They were then exposed to bromadiolone baited plots 1, 5, 3 and 6 months after bait application, trapped during the 7 to 10 days following their re-introduction and bromadiolone residues were measured in their tissues. The week before re-introduction, the voles already present in the treated plot (i.e., potentially surviving or naturally recolonizing individuals) were trapped in order to avoid competition with the ones we re-introduced. 1.5 months after the treatment, 75% of the re-introduced voles (68) exhibit bromadiolone residue in the liver, the concentrations (median: 2 ng/kg) being low compared to those measured by Sage et al. (2008) STOTEN 407 in voles trapped during the days following bait application. Fifty eight % of the individuals re-introduced 3 months after the treatment were exposed to bromadiolone (7/12), liver residues were also globally low with a median of 1.6 mg/kg but one vole exhibited a concentration of 25 mg/kg that was a constituting a soil point by Sage et al. around. Some of these individuals were trapped just after a treatment. Six months after bait application in the experimental plot, no re-introduced voles were exposed to bromadiolone while, among the 7 voles trapped the week before the re-introduction, 2 have very low bromadiolone concentrations in the liver (<1 mg/kg). Our results show that bromadiolone may persist in vole populations during several months after a treatment and thus, may lead to chronic exposure of wildlife to low doses of rodenticides.

TU082
Temporal variations of wood mouse exposure to Cd and Pb in a smelter-impacted area and relationships with diet
C. Fritsche, CNRS / UMR ChronoEnvironment; F. Raoul, University of Franche-Comté / CNRS / UMR 6249 Chrono-environment; M. Coeurdassier, University of Franche-Comté / UMR ChronoEnvironment; N.W. van den Brink, Wageningen University / Dept of Toxicology; R. Scheiffer, University of Franche-Comté / ChronoEnvironment
Although levels of pollutants accumulated in organisms and related deleterious effects may vary in time, relatively few investigations have been made. The present study investigated inter-annual and inter-seasonal variations in exposure to terrestrial animals chronically exposed in metal polluted environments, and the temporal trends of metal transfer in food webs after termination of atmospheric emissions from point sources such as smelters. The aim of this work was to study temporal trends of exposure of the wood mouse Apodemus sylvaticus, an omnivorous rodent, to cadmium (Cd) and lead (Pb) at a case study in the area of Jonage, a small smelter that closed down in 2003. In investigating inter-annual and inter-seasonal differences in accumulation, the goals were to test whether ageing of soil pollution may decrease wood mice exposure, whether exposure was greater in autumn as observed for some other pollutants, and whether temporal variations may be related to changes in the diet. The sample sizes were 116, 88, 73, and 35 individuals in the years 2006, 2007, 2008, 2010 and 2013, respectively, with 191 captured during autumn and 196 during springtime. Concentrations of Cd and Pb were measured in liver and kidneys for exposure assessment. Samples of mouse muscles, and potential diet item (grass, leaves, seeds, berries, above-ground invertebrates, earthworms) were collected for measurements of carbon and nitrogen stable isotopes in order to assess the diet. Preliminary results showed both inter-annual and inter-seasonal variations of metal accumulation, with different patterns for Cd and Pb. The relationships between hepatic Cd and soil Cd did not differ between seasons but between years, concentrations in tissues increasing with soil pollution until 2008 but decreasing in 2010 and 2013, suggesting a decrease of Cd transfer in food webs. Conversely, the relationships between hepatic Pb and soil Pb did not vary between years for a given season. These variations were likely due to differences in diet. Measurements of stable isotopes are ongoing. Further analyses will be conducted to investigate the temporal patterns of metal levels in kidneys, check whether temporal variations were site-specific, and relate the differences observed with diet.

TU083
Population vs individual biomarkers in ecotoxicology: Exploring this gap in preserved sites from A. Sánchez-Chávez, Autonomous University of Barcelona / Microscopy Facility; M. Massa, Autonomous University of Barcelona / Animal Biology Vegetal Biology and Ecology; F. Muñoz-Muñoz, Universitat Autònoma de Barcelona / Departament de Biologia Animal
Small mammals have been often checked as bioindicators of environmental quality in ecotoxicological studies. Such studies have been usually focused on biomarkers from molecular to individual levels, whereas population effects have been scarcely evaluated. The aim of the present work is to quantify individual and population measures of asymmetry (total, fluctuating asymmetry or FA), and directional
asymmetry or DA) in shrews and compare them with metal levels in tissues. We quantified potentially toxic metals in main target tissues of 179 specimens of white toothed shrew Crocidura russula from 4 different localities in the Iberian Peninsula (Cers, Garraf, Doñana, and Aljustrel), 2 of them being protected areas (Parc Natural del Garraf and Parque Nacional de Doñana). Within each locality, animals were trapped in 2 different sites, one polluted and one non-polluted. Thus, in addition to the ecotoxicological implications of studied localities, the polluted sites were selected for the records of the pollution source (a coal-fired power plant in Cers, a landfill in Garraf, and 2 pyrite mines affecting Doñana and Aljustrel). The measures of asymmetry were obtained analyzing by means of geometric morphometrics the shape and the size of the two mandibles of each individual. Our results showed a general trend of increased levels of metals in animal tissues and of pollutant content in target tissues (Pb, Cd, Fe, Cu, Co, Cr), which could indicate that the presence of parasites in the animals induce asymmetry as a bioindicator of environmental contamination in Iberian hare (Lepus granatensis). For this purpose the physiological levels of the oxidative stress biomarkers as malondialdehyde (MDA) and reduced glutathione (GSH) in liver and kidney of Iberian hare need to be considered, as well as the correlation of these biomarkers, type of hunting or parasitism on the levels of these biomarkers. Hares (34 males and 37 females) were sampled from two types of hunting: shooting (42) and hare coursing with greyhound (29). Livers and kidneys were collected and once examined for the presence of parasites were frozen (~80°C) until determination of MDA and GSH, which were assayed using the Recknagel et al. (1982) and Hissin and Hilf (1976) methods, respectively. Liver of parasitized hares showed higher levels of MDA and lower levels of GSH than non-parasitized ones. In addition, animals hunted with greyhound showed lower levels of GSH than animals shot. These results could indicate that the presence of parasites in the animals induce oxidative stress in liver, which is also observed when hunting with greyhound as indicated by the GSH depletion. In kidney, male hares showed higher levels of MDA and lower levels of GSH, which could be due to the effect of feeding the hares with different kinds of food. Female hares showed lower levels of MDA than animals hunted with greyhound what could be explain by the stress caused by the coursing. Then, although oxidative stress parameters could be used as biomarkers in environmental studies with hares as bioindicator, the type of hunting and the presence of parasites are factors that should be taken into consideration when interpreting their levels.

TU085 Organochlorine contaminants (pesticides and PCBs) in red deer: levels and public health J. Vázquez, J. Benavides, M. Pérez López, E. Soler, University of Extremadura / Faculty of Veterinary Medicine Game animal are used as bioindicators of environmental contamination in the places where they live due to the fact that they can accumulate organic pollutants in their tissues. Game is consumed by population and can be a way to increase the burden of contaminants in consumers, being a public health concern. The aim of this work was to study the levels of organochlorine pesticides and polychlorinated biphenyls (PCBs) in the adipose tissue of red deer (Cervus elaphus) from Extremadura (West of Spain), and their environmental and public health implications by analyzing a total of 46 individuals. Samples were obtained from 6 different hunting areas, and for each deer a sample of perirenal fat tissue was taken. Lipids were extracted with petroleum ether and cleaned-up with sulphuric acid. The final extract was analyzed by gas chromatography with mass detector (GC/MS) in NCI mode. The detected chlorinated pesticides and their frequency of occurrence (%) were: aldrin (11 %), heptachlor (17 %), α-HCH (20 %), endosulfan sulfate (28 %), chlorides (50 %), HCB and heptachlor epoxide (67 %), γ-HCH (70 %), β-HCH and DDT (87 %) and DDE (100 %). The detected PCBs were PCB 28 (2 %), PCB 52 (9 %), PCB 118 (33 %), PCB 138 (72 %), PCB 180 (78 %) and PCB 153 (85 %). The highest mean level was that from DDE (249.65 ng/g), much higher than those from the other detected compounds. There were statistically significant differences between hunting areas only for beta-HCH between 2 areas, and for DDTs between one area with respect to the others. For PCBs, a regular distribution between sampling sites were also observed, and only a statistically significant difference was found for PCB 180 (30%) to 28% in the studied areas and levels detected in this study are similar to those reported in other parts of Spain and other countries for red deer, indicating not very different environmental status in hunting areas with respect to these compounds. The number of samples that exceeded the maximum residue limits (MRLs) established in the EU by Regulations (EC) 396/2005 and 1881/2006 was: 2DDT (2 samples), β-HCH (1 sample), γ-HCH (3 samples) and 2 PCBs (4 samples). No legal LMRs has been specifically established for these compounds in game animals. The much more lower production and consumption of game meat respect to domestic animals support the conclusion that red deer meat poses no risk to consumers.

TU086 Mercury in golden eagle eggs - use of stable isotope to distinguish dietary influences in long-term monitoring studies R.F. Shore, Centre for Ecology & Hydrology (NERC); L.A. Walker, H.K. Grant, Centre for Ecology Hydrology; E. Potter, Centre for Ecology and Hydrology; M.M. Pereira, CEH Fundamentals of mercury (Hg) emissions may rise, in part through increased coal-fired power generation, but the recent United Nations Environment Programme Minamata Convention on Mercury aims to control anthropogenic releases of Hg to the environment. Long-term future trends in environmental Hg concentrations are therefore uncertain. One way of assessing such trends is to monitor accumulation by sentinel wildlife. We are investigating the potential of using added eggs from northern golden eagles (Aquila chrysaetos) to detect change and environmental bioavailability and bioavailability in the uplands of Britain. To date, nests have been defined as "inland" if >3 km from the coast but golden eagles have variably-sized territories and such classification is somewhat arbitrary. So some so-called "inland" nesting birds may feed partly or extensively on marine fauna, such as seabirds. We have found previously that egg Hg concentrations differ between nests that are 3 km from the coast, likely reflecting differences in terrestrial and marine Hg transfer pathways. If our current classification of nests as "inland" is inaccurate, dietary (terrestrial vs marine) influences on Hg accumulation may obscure detection of future changes in Hg accumulation arising from altered upland deposition. We examined stable carbon (δ13C), nitrogen (δ15N) and sulphur (δ34S) isotope values on eggshell (10 eggs) to detect change in diet. We found no clear patterns for δ13C and δ15N but δ34S values in eggs from nests (Haliaeetus albicilla) eggs from the same area. This suggests that female golden eagles from nests within 3 km of the coast had a significant marine component to their diet. We defined 95% confidence limits (CLs) for δ34S for those eggs. We then examined δ34S values for eggs from nests >3 km from the coast and found that 33% had δ34S values within the CLs for coastal nests, suggesting that females from a third of supposed inland nests were largely feeding on marine prey. Our results highlight the need for stable isotope measurements (especially δ34S) to identify those eggs that truly reflect Hg derived from upland terrestrial diet. Such discrimination is essential if golden eagle eggs are to be used as a biomonitor of change in Hg deposition and bioavailability in upland Britain.

TU087 Assessment of exposure and effects on stress response of perfluorooctyl and polyfluoroalkyl substances in White-tailed eagle and Northern goshawk nestlings using non-invasive samples P. Gómez-Ramírez, Sociosanitarias Sciences; J. Busnes; T. Johnsen, Norwegian Institute for Nature Research; D. Herke, NILU - Norwegian Institute for Air Research / MILK; G.S. Eggen, Norwegian University of Science and Technology / Department of Biology; V.L. Jaspers, Norwegian University of Science & Technology / Biology Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a large group of compounds widely used in industrial processes because of their thermal stability and resistance to degradation and low metabolism, making most of them are nowadays ubiquitous. In addition, several toxic effects have been linked to these compounds. For example, perfluorooctanoic sulfuric acid (PFO) can displace corticosterone (the main stress hormone in birds) from their binding globulins in blood, causing an increase of free corticosterone, readily able to stimulate gluconeocorticoid and androgen receptors in birds. In order to evaluate the influence of PFASs on stress response in birds of prey from Norway, corticosterone in plasma and feathers were analysed in 14 nesting White-tailed eagles (Haliaeetus albicilla) and 11 nesting Northern goshawks (Accipiter gentillis) using an ELISA method. PFASs were analysed using UHPLC-MS/MS in plasma and feathers of the same White tailed eagles while they could only be analysed in the plasma of 10 Northern goshawks due to too low sample availability of feathers. Mean δPFAS were higher in plasma (White-tailed eagles mean δPFAS=31.5 ng/mL, Northern goshawk mean δPFAS=19.05 ng/mL) than in feathers (δPFAS=12.97 ng/g). Although PFOSA was not found in any plasma sample, it was detected in all the feathers. This was also previously found in White-tailed eagles from Norway and Greenland and could
indicate either that the feathers/preen oil are a route for excretion of PFOSA or that there is external contamination in feathers. PFAS levels in plasma of both species were up to 10 times lower than in Bald eagles (Haliaeetus leucocephalus) nestlings from the Upper Midwestern United States, but similar to nestlings of White-tailed eagles and Goshawks studied in Norway between 2008 and 2010. Also coinciding with the studies in birds of prey from Norway, levels were higher in White-tailed eagles than in Goshawks. XPFAS seem to cause an increase of free plasma concentrations in our study, based on a positive correlation. However, according to in vitro studies in Bald eagles, our concentrations would be too low to cause a displacement of corticosterone from binding globulins. Moreover, the individuals from our study are not considered at risk for PFOA as their levels in plasma were hundreds to thousands times lower than the toxic reference values in Bald eagles (250–4000 ng/ml). Acknowledgements: NLS Science and Sustainability.

TU088

Levels of perfluorinated compounds (PFCs) in northern gannet (Morus bassanus) eggs from two UK colonies

M.G. Pereira, Centre for Ecology & Hydrology / Lancaster; S. Lacorte, IDAEA-CSIC / Environmental Chemistry; E. Potter, Centre for Ecology and Hydrology; L.A. Walker, Centre for Ecology & Hydrology; R.F. Shore, Centre for Ecology & Hydrology (NERC)

We measured PFCs in eggs of the marine sentinel species, the northern gannet (Morus bassanus) from two Scottish colonies (Ailsa Craig in the Eastern Atlantic and Bass Rock in the North Sea). Eggs were collected as part of the UK Predatory Bird Monitoring Scheme (PBMBS – http://pbmbs.ac.uk). In this preliminary work, we used LCMS-MS to analyse 13 perfluorinated carboxylic PFCAs and 4 perfluorinated sulfonates (PFSSs) in eggs collected in 2007. Of the compounds analysed, seven PFCAs and two PFSSs were detected. In Ailsa Craig, the sum of the PFCAs ranged between 6.8-20.8 ng g⁻¹ ww and of the PFSSs between 40.8 - 73.3 ng g⁻¹ ww, except in one egg with PFSS concentrations of 206.6 ng g⁻¹ ww. In Bass Rock, we measured a sum of both PFCs and PFSSs varying between 16.5-38.7 ng g⁻¹ ww and 28.0-85.3 ng g⁻¹ ww, respectively. We found no significant differences between colonies. As expected, PFOs (Perfluorooctanesulfonic acid) accounted for the majority of the PFSSs (98% and 99%). The most abundant PFCAs were perfluorotridecanoate and perfluoroundecanoate in both colonies. Perfluorobutanoate and perfluorononanoate were the only two compounds with concentrations significantly higher in eggs from Ailsa Craig than from Bass Rock. From this limited data set it appears that gannet eggs from both colonies contain relatively low concentrations of PFCs compared to concentrations reported in the literature. Concentrations of PFOS were lower by an order of magnitude of residues associated with adverse effects, except for one egg from Bass Rock where these levels were exceeded. The majority of eggs from both colonies contained PFOS residues that exceeded a suggested permitted no effect concentration (PNEC) for this compound.

TU089

Do parental feeding strategies affect early offspring exposure to mercury contamination in Lesser black-backed gulls (Larus fuscus)?

C.S. Santos, Ghent University (UGent) / Terrestrial Ecology Unit TERECE Department of Biology; L. Blondel, Ghent University / Terrestrial Ecology Unit Departament of Biology; M. S. Monteiro, Aveiro University / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; S. Loureiro, Universidade de Aveiro / Biology; L. De Neve, L. Lens, Ghent University / Terrestrial Ecology Unit; M. C. Parreira, Department of Biology and CESAM; S. Loureiro, University of Aveiro / Biology; J. Collins, R. Scantlebury, C. Castro, H. Saxton, Westminster College / Biology; J. Collins, R. Scantlebury, C. Castro, H. Saxton, Westminster College / Biology; C.M. Stracey, Guilford College / Biology; F.J. Black, Westminster College / Chemistry

Mercury (Hg) is a neurotoxin and global pollutant that in aquatic ecosystems is converted into methylmercury (MeHg), the chemical form that is readily biomagnified up food chains to toxic concentrations. The Great Salt Lake (GSL) in Utah contains some of the highest MeHg concentrations ever measured in natural waters. While terrestrial organisms typically have very low concentrations of Hg because MeHg is produced almost exclusively in aquatic environments, the Hg concentrations we have measured in spiders along the shoreline of the GSL are 60 fold greater than in spiders at other sites in the surrounding terrestrial ecosystems where they are eaten by spiders and other organisms. We also hypothesized that songbirds that consume spiders would be exposed to high levels of Hg that could cause adverse effects. To test these ideas we characterized spatial and temporal variation in total mercury (HgT) and MeHg concentrations in surface water, brine flies, and spiders at the GSL in 2012-2013. We also quantified spatial distribution of HgT concentrations during the 2012-2014 breeding seasons in blood of adult and nestling Loggerhead Shrikes, Lanius ludovicianus, a terrestrial songbird of conservation concern, and investigated sublethal effects due to Hg exposure. Concentrations of HgT were roughly 100-700 ppb (dw) in brine flies and 400-5000 ppb (dw) in spiders. Seasonal maxima in concentrations of HgT and MeHg in flies are observed in spring and fall, periods of peak migratory bird numbers at the GSL. These seasonal trends mirrored HgT concentrations in surface waters of the GSL, suggesting the lake was the source of mercury. HgT concentrations in nestling shrike blood averaged 91 ppb (ww) and ranged from 7-354 ppb. Average HgT concentrations in adults/juveniles was 912 ppb and ranged from 127-4003 ppb. Approximately 20% of adults/juveniles had blood HgT concentrations above thresholds previously shown to reduce breeding success in other songbirds. HgT concentrations of shrikes decreased with increasing distance from the shoreline, again suggesting the GSL is the ultimate source of HgT in these organisms. Preliminary data from a subset of videos do not show a correlation between nestling HgT levels and nestling feeding rates. Ongoing research will attempt to correlate HgT concentrations and additional parental care behaviors.

TU092

Assessment of embryotoxicity, post-hatching development, and long-term effects on behavior and reproduction following in ovo exposure to methylmercury in zebra finches (Taeniopygia guttata)

M. Yu; M. Eng; T. Williams; J.E. Elliott, Environment Canada / Science Technology Branch

Mercury is a toxic heavy metal and common environmental contaminant found in terrestrial and aquatic environments as inorganic mercury and as organic methylmercury (MeHg). MeHg is considered to be the most toxic. Field and captive avian dosing studies have reported adverse reproductive effects from MeHg exposure. Many studies have been done on the effects of MeHg exposure via maternal transfer, and in ovo and perinatal exposure in waterbirds and poultry; however the effects of MeHg in passerine birds is less well understood. Also, avian studies have provided evidence that MeHg may cause adverse neurological and
behavioural effects. The objectives of the current study were: (1) to assess embryotoxic effects of in ovo exposure to MeHg; and (2) to assess post-hatching effects of in ovo exposure to MeHg on survival, development, mating behavior, and reproduction in a model songbird species, the zebra finch (Taeniopygia guttata). To assess the embryotoxic effects of in ovo exposure to MeHg, zebra finch embryos were exposed to MeHg via egg injection. Either a vehicle control (filtered ddH2O), a 2 g/L of a high dose of MeHg (3.2 μg Hg/g egg), or a low dose of MeHg (1.6 μg Hg/g egg) was added to the incubation medium. The following endpoints were assessed: egg hatchability and number of live hatchlings; effects on hatching success, chick growth, fledging success, and hematology (i.e., blood samples collected at 15 and 30 days of age). There was an effect of dose on overall hatching success, with decreasing hatching success in a dose-dependent manner. However, chick growth, body mass, and hematocrit were not affected by MeHg exposure. In order to study potential long-term effects of embryonic exposure to MeHg, surviving chicks were raised to sexual maturity to assess potential adverse effects to male courtship behavior and female reproductive performance. Embryonic exposure to MeHg did not appear to have long term, neurotoxic effects on male courtship behavior or song quality. This study is in progress, and in vitro exposed female zebra finches are undergoing breeding trials to assess effects on reproduction. There were no treatment differences in clutch size. Effects on hatching success, fledging success, and offspring growth are currently being assessed. The results gathered thus far would suggest that embryonic exposure to MeHg has acute effects on survival, but no overt long-term effects on growth, courtship behaviour, or reproductive performance.

TU093 Biological responses of the "Rana Criolla" (Leptodactylus latrans) inhabiting a sewage-effluent receiving aquatic ecosystem of Argentina


In Argentina, sewage effluents are discharged on aquatic ecosystems raw or poorly treated. These bioeffluents can become conduits for pollutants reaching the receiving waters. The objective of the present study is to assess the potential biological effects induced by sewage effluents on wild populations of the Rana Criolla frog (Leptodactylus latrans) inhabiting receiving aquatic ecosystem. Ten organisms (five females and five males) were collected either from the Girado stream (receiving the primary treated effluent) or from La Plata City. External and internal abnormalities were inspected together with the assessment of the condition factor (K), the hepatosomatic (HS) and gonadosomatic (GS) indexes. In addition, catalase (CAT), ethoxy-resorufin-O-deethylase (ERO), benzoyloxy-resorufin-O-dealkylase (BROD) activities together with total protein (PROT) and diisobutiric acid reactive substances (TBARS) were assessed in the liver. Internal malformations were observed in 80% of the frogs from the Girado stream and none from the control site, characterized by swollen soft tissue along the backbone. In addition, significant (p=0.00001) reduction in the GSI (35%) was observed in the females frogs at the Girado. No differences were observed for K or male GSI. A significant (p=0.028) increase of BROD activity (62%) was observed in Girado frogs. EROD was significantly (p=0.042) higher in males than females (7.9 μg AHH/mg protein) and different among populations showing a sex/site interaction effect (p=0.043). CAT and TBARSes were not statistically different among populations, but showed the same trend than BROD contributing to the same factor in the PCA analysis. In summary, frogs living under the sewage effluent influence showed internal abnormalities and less development in female gonads together with a disruption of the EROD activity in the males, an increase of phase I detoxifying enzymes BROD activity accompanied by a sublethal enhancement of the antioxidant system and increase of the lipid peroxidation. All these evidences, show a clear impact of the sewage discharges on the health of Rana Criolla and probably other wild amphibian species inhabiting receiving aquatic ecosystems.

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TU094 Environmental impact assessment of chemical pollution in a Spanish natural reserve: a study of water quality

Fluctuating energy intake through dietary restriction alters basal and induced levels of P-glycoprotein-mediated xenobiotic efflux activity in Rainbow trout (Oncorhynchus mykiss)

C.J. Kennedy, Simon Fraser University / Department of Biological Sciences

Teenage teleosts can be exposed to natural conditions of fasting and starvation, often during over-wintering on an annual basis in temperate climates. In addition to natural periods of limited energy, exposure to chemical stressors can increase energy demands and may result in trade-offs that could affect the success of the organism, including those related to limited energy conditions. The energetic costs of basal and induced levels of P-glycoprotein (P-gp/ABC1)-mediated, ATP-dependent efflux of xenobiotics as a primary line of cellular defense against exogenous compounds (multixenobiotic resistance, MXR) is currently unknown. In this study, P-gp activity as well as oxygen consumption (as an indicator of energy use) were examined under four treatment conditions: 1) fed fish, 2) fasted fish (12 weeks), 3) fish fed and P-gp induced (clotrimazole 10 mg/kg g), and 4) fasted fish and P-gp induced (clotrimazole 10 mg/kg g). Twelve weeks of fasting reduced the basal activity of P-gp compared to fed fish. Chlortrimazole increased P-gp activity in fed fish and fasting did not alter the maximum induction of P-gp by chlortrimazole. However, the increase to maximum induction was slower in fasted fish compared to
fed fish. Oxygen uptake 12 weeks in fed or fasted fish exposed to a P-gp substrate (Rhodamine 123) only or induced with clotrimazole only were not affected, however, fed or fasted fish with induced P-gp levels and then exposed to the P-gp substrate exhibited increased respiration rates. In summary, prolonged fasting affects the ability of fish to efflux xenobiotics, and in addition, when P-gp is operating at maximum levels, energetic costs may be substantial.

TU097 Toxicity Assessment of Ashes from Tropical Savanna Wildfires to Aquatic Biota
D. Brito, University of Brasilia / Biology; C.J. Passos, University of Brasilia; E.C. Oliveira-Filho, Z. Malena, University of Brasilia
Flammability and impacts in the role of LDN in tropical savannas, meanwhile climate change scenarios predict an increase in the frequency and severity of wildfires worldwide, mainly in those zones. The effects of forest fires on vegetation cover, catchment erosion and their detrimental effects on different environmental matrices have been well documented, however very few studies have examined the potential ecotoxicological effects of wildfire on aquatic organisms in tropical zones. We investigated the effects of ash from three different areas (two native vegetation areas and a pasture area - a landuse type very susceptible to wildfires in Brazil) through ecotoxicological bioassays using three aquatic species from distinct trophic levels: microcrustacean Ceriodyaphia dubia, fish Danio rerio and the mollusc Biomphalaria glabrata. Ashes (leaves, wood and bark) were collected in burnt areas, prepared the solution (100gL\(^{-1}\)) in culture water and mixed for 90hrs for distillation (5.12–110°C). The only declined. These factors likely contributed to this decline, including overfishing, climate change and obstacles to migration, but recent studies suggest that contamination also plays a role. The objective of this study was to examine the relationships between eel contamination and a range of enzyme indicators of various metabolic capacities. To this end, European yellow eels were sampled in two clean and two contaminated sites in France and the same design was repeated in the province of Québec (Canada) for American eels. We measured the concentrations of inorganic contaminants (Ag, As, Cd, Cr, Cu, Hg, Pb, Ni, Se and Zn) in liver and muscle and of organic contaminants (Sum of OCPS, sum of PCBs, and sum of PBDEs) in muscle. As indicators of aerobic capacities, we measured the activities of the mitochondrial enzymes citrate synthase (CS) and cytochrome C oxidase (CCO), while the activity of lactate dehydrogenase (LDH) was used as an indicator of anaerobic capacities and that of pyruvate kinase (PK) served to estimate glycolytic capacities. We also measured glucose-6-phosphate dehydrogenase (G6PDH), involved in lipid and antioxidant metabolism. In A. rostrata liver, CS activity was strongly and positively correlated with Pb contamination, while negative correlations were observed between G6PDH and PCBs and between CCO and Hg concentrations. Activities of both PK (+), CS (-) and CCO (-) and Hg concentrations were observed. Also, a positive correlation was found between PK and PCBs, while strong negative correlations between both CS and CCO were observed with OCPS, PCBs and PBDEs. In A. anguilla liver, PK was positively correlated and CCO negatively correlated with Pb concentrations. In muscle, PK and LDH activities were negatively correlated with As and Se respectively. Finally, CS, CCO and LDH did not respectively negative correlations with PCBs, PCBs and PBDEs. Overall, our study suggests that several of the contaminants investigated affect metabolic capacities of yellow eels of both species. Given the importance of aerobic and anaerobic capacities for locomotion, feeding and migration, the results of this investigation add to a growing body of evidence that environmental contamination plays a role in the decline of Atlantic eels.

TU099 Aromatase and vitellogenin mRNA expression in an endangered fish species (Anguilla Anguilla) from the Loire estuary (France) : relationships with PCB contamination
I. Blanchet-Letrouve, I. poirier, EMS EA; A. ZALOUK-VERGNOUX, Université de Nantes; A. Lafont, Muséum National d’Histoire Naturelle; S. Balcho, Muséum National d’Histoire Naturelle; S. Dufour, Muséum National d’Histoire Naturelle / UMR BOREA; C. Mouneyrac, Université Catholique de l’Ouest / EMS EA Estuarine zones are extremely fragile due to increasing stress from anthropogenic activities among those. The Loire estuary (France) is potentially exposed to various contaminants including Endocrine Disruptors Compounds (EDCs) able to impact the reproduction physiology of fish. Even if the European eel (Anguilla anguilla) is not the most suitable sentinel species among Teleosts, this study aimed to investigate whether it may still be the target of estrogenic disruption, which could contribute to the decline of this species. Among the different molecules involved in reproductive function, vitellogenin (VTG) and aromatase (Arom) are of great interest because alterations in their expression could sign disruptions in sexual development. Quantitative real-time Reverse Transcription Polymerase Chain Reaction (q RT PCR) was used in this study to amplify responses of hepatic Vg and Arom transcripts in liver. European eels (A. anguilla) were sampled in May 2009 (N=57) and November 2010 (during the downstream migration, N=10) in two sites of the Loire estuary (upstream: Varades; downstream: Nantes). Reproductive (gender, sexual maturity stage) and biometric parameters of collected eels were determined. While only female silver eels should express hepatic Vg mRNA, abnormal levels were also detected in a large proportion (38%) of the other individuals: undifferentiated, yellow females, yellow and silver males. The principal component analysis performed between biometric parameters, Vg and Arom mRNA expression and further their populations. The objective of the present study was to investigate potential metabolic correlations during the sexual maturation stage, neither to the levels of PCBs. Arom mRNA seemed to occur in the early stage of gonadal maturation and was detected in the liver of 12 out of 67 organisms. Its expression was correlated to the dl and nld-PCB of the muscles expressed in lw. Further investigations on other environmental estrogenic EDCs are needed in order to explain the unusual Vg expression.

TU100 Fate of PCBs during developmental and sexual maturation of European eel (Anguilla anguilla) F. Picon, M. Elie, C. Trifuoggi, University of Naples Federico II / Biology; C. Herrenknecht, University of Nantes / EMS EA; S. Dufour, Muséum National d’Histoire Naturelle / UMR BOREA; B. Le Bizec, ONIRIS / LABERCA; C. Mouneyrac, Université Catholique de l’Ouest / EMS EA; I. poirier, EMS EA Among estuarine fish species, European eel (Anguilla anguilla) is embryonic and reared as an endangered species in Aquaculture units. The objective of the present study was to investigate potential metabolic correlations during the silvering process comparing the PCB contamination levels in immature organisms such as glass eels and yellow eels in and potential genitors. PCB contamination levels of both gonads and muscles of yellow and silver eels from the Loire estuary have been compared in function of age class (6-10 years; >10 years). The immature yellow gonads appeared two fold higher contaminated than the muscles for dl and nld-PCBs (expressed in wet weight (ww) and in dry weight (dw)) in yellow eels belonging to the age class 6-10y. This result could be explained by the lipid weight of the gonads. For silver eels belonging to the 6-10 and 10-13 age classes, nld PCB levels of gonads were significantly higher than those of muscles whatever the unit expression. Moreover, strong correlations between PCB levels of gonads and muscles in silver eels expressed in lw were found for each analyzed congener (except for PCB 81), whereas no correlation was found in yellow eels. These results highlighted significant correlations between the gonad and muscle lipids during the growing phase of silver eels. A mean contamination level of 18.5 ng TEQ kg\(^{-1}\) of gonad expressed in ww was obtained, suggesting a potential risk of endocrine disrupting activities. The PCB contamination was different between individuals belonging to the different developmental stage and matrices. The non metabolizable congeners 126 and 169 seemed particularly associated to the silvering process. Their constancy in glass eels could also sign a exchange of these contaminants during the hatching, from oocyte yolk to embryos, suggesting a materno foetal transfer.

TU101 TOXICITY OF IMPROPERLY DISCARDED PHOTOVOLTAIC PANELS R. Panzuco, Biologia; B. Avallone, University Federico II of Naples; R. Cerciello, University of Naples Federico II / Biology; V. Mazzella, University of Naples Federico II / Biologia; M. Trifuggi, University of Naples Federico II / Scienze chimiche; C.M. Motta, University Federico II of Naples / Department of Biology The exploitation of solar energy is considered one of the possible solutions to the increasing energy demand and for this reason the use of photovoltaic cells (PVC) has progressively increased in the last thirty years. First commercially available PVC have been installed in the early 80; they are now at the end of their life and need to be disposed, a costly procedure due to the potential toxicity of their
components. The purpose of this work was to assess the environmental risk associated with an incorrect disposal of PVC and, in particular, to verify if damaged panels release in water components harmful to the aquatic environment. For this purpose, fragments of PVC panels were kept in infusion in water for 30 days. The resulting solutions were analyzed to determine chemicals released and to assess toxicity on Daphnia magna, Artemia salina and sea urchin (Paracentrotus lividus). Results demonstrate that the panels, if damaged, release different metals (especially arsine) and aldehydes (Formaldehyde and acetaldehyde) that experimentally examined. In particular in the two crustaceans PVC solution increase mortality and induces cytological damages in the naupliar eyes. In P. lividus, PVC solutions alter normal progression of embryo development, causing asynchrony and producing amorphous plutei. In conclusion, data obtained indicate that it is essential to set up programs for safe disposal of photoelectric panels.

TU102
Toxicological effects of herbicide Raundup® and insecticide Fastac® on aquatic organisms
M. Balode, R. Medne, A. Mednis, L. Alegretti, LEAL Laboratory of Ecotoxicology
Due to the intensive farming all around the world, the usage of pesticides increase significantly in the last two decades. Consumption of pesticide combined with potentially high toxicity level is a huge threat to the wildlife. Acute toxicity of two commonly used pesticides - the pyrethroid insecticide Fastac with active substance alpha - cypermethrin and glyphosate based herbicide Raundup was detected. Laboratory cultures of different trophic level organisms (microalgae, zooplankton and fish) - green algae Desmodesmus communis, crustaceans Daphnia magna and fish species Poecilia reticulata were used as testobjects. No-observed effect concentrations (NOEC), the lowest observed effect level concentrations (LOEC) and EC50 were detected using Standard Methods for Acute Toxicity Tests for freshwater algae, invertebrates and fish. The effect of recommended spraying concentrations (15ml/l for Raundup; 1,5ml/l for Fastac) were estimated as well. Growth inhibition and mortality were used as parameters for the detection. Impact studies show high sensitivity of freshwater algae Desmodesmus communis - to the presence of herbicide Raundupand crustaceans Daphnia magna - to insecticide Fastac. Test organisms arranged from more sensitive to less one in following order: for herbicide Raundup - Desmodesmus communis, Daphnia magna, Poecilia reticulata, but for insecticide Fastac – Daphnia magna, Poecilia reticulata, Desmodesmus communis. Beside that Raundup and Fastac showed high toxicity level also for non target organisms (insecticide - to algae; herbicide - to crustaceans and fish), meaning that tested pesticides may pose an unacceptable risk to the aquatic environment. The study was financed by ESF Project "Creation of a new scientific group for modernization of aquaculture technology" project Nr. 201300671/D1P1/1.1.2.0/13//APIA/VIAA/060 (2014-2015).

TU103
Age-specific and time-specific life tables of natural and lab Parhawa hawaiensis populations as potential endpoints for long-term effects of toxic agents
B. Drizaus, L. Alegretti, LEAL Laboratory of Ecotoxicology and Environmental Microbiology School of Technology; G.d. Umbuzeiro, FACULTY OF TECHNOLOGY -UNICAMP / LEAL, M.N. Flynn, LEAL Laboratory of Ecotoxicology and Environmental Microbiology School of Technology Life table parameters and reproductive fitness parameters are being used as relevant tool to measure of ecological impact. The aim of this study is to describe the life characteristics and reproduction potential of natural and lab Parhawa hawaiensis through the construction of age-specific life-table for a natural population and time-specific life-table following a cohort for lab culture. The construction of age-specific life table of a natural population of P. hawaiensis input data for the determination of age classes and fecundity were obtained following the procedure: 12 monthly samples from an algae belt in Itanhaem Beach - SP – Brazil were taken; individuals were screened for identification, sex determination, size, measurement, and eggs count. Each sample provides a life table that is taken in account for production of mean parameters such as intrinsic growth rate (r), reproductive potential (R0) and generation time (T). (r = ln (R0)/T). Data for the construction of time-specific life table of a cultured population of P. hawaiensis was obtained through the follow-up of a cohort (n = 10) extracted from 10 females in the same time interval brood spawn in laboratory conditions. Following the growth and development of each individual and analyzing the cohort decay, we undertook a number of oviposition choice experiments to compare water treated with and without dye at the concentrations specified by the manufacturer. Captive gravid Culex pipiens female mosquitoes (wild caught and laboratory strains) were presented with a choice of containers with or without dye in a field, semi-field (wild caught in a tent) and laboratory conditions. Our preliminary results show that there is a significant difference between the treatments. The black dye is attractive to gravid mosquitoes, encouraging them to lay eggs preferentially in dark waters. Since these mosquitoes are known to be competitors of cladocerans and other macroinvertebrates, the dye is possibly altering the community composition in ponds. Although the pond dye is not exerting a direct chemical stress on the community, it is a potential engine of community change.

TU104
Species-related changes in gene expression after 17α-ethinylestradiol (EE2) exposure in water snails
C. Hultin, Lund University / Lund University Centre for environmental and climate research; A. Persson, Lund University; M.C. Hansson, Lund University / CEC The endocrine disruptive chemical (EDC) 17α-ethinylestradiol (EE2) is today a widespread problem in the environment due to its high release and tendency to bioconcentrate in aquatic organisms. It is known that low levels of EE2 can affect both growth and reproduction of gastropods. Moreover, gastropods are considered sensitive to environmental changes, causing many aquatic snails to be endangered. Here, we will identify new information about how EE2 exposure affects aquatic snails at the gene expression level. This will enhance our general understanding of how human-derived endocrine disrupting compounds (EDCs) can interfere with endocrine-related genes of gastropods. This study is part of a long-term research collaboration that has previously shown how EE2 exposure can interfere with somatic growth of the aquatic natural snail Bithynia tentaculata. However, another natural water snail (Radix balthica) was unaffected by the treatment, indicating species-related differences of how a presence of EE2 affects growth. Here, we aim to investigate species-related consequences of EE2 exposure at the gene-level in B. tentaculata and R. balthica. Two gastropod species (B. tentaculata and R. balthica) were collected from a natural population in southern Sweden. By using before-and-after impact design, we exposed B. tentaculata and R. balthica snails to nominal concentrations of 10 and 100 ng/L EE2 at our laboratory during 24 hours and/or 72 hours. We exposed natural B. tentaculata and R. balthica snails to nominal concentrations of 10 and 100 ng/L EE2 at our laboratory during 24 hours and/or 72 hours. More specifically, we test if a species difference in growth also is reflected at the genetic level in terms of gene expression of the estrogen receptor (ER) genes. Our analysis show a significant change in the B. tentaculata erα gene expression and not in the R. balthica after 72 h of EE2 exposure (ANOVA, p<0.05, n=6-13, prel. data), which is coherent with our previous growth analysis of these species after EE2 exposure. Ongoing studies will try to further unravel the links between growth (population effect) and endocrine-related genes (molecular biomarkers) in gastropods.

TU105
The effect of pond dyes on mosquito colonisation of water bodies
N. Ortiz, University of Reading / School of Biological Sciences; A. Callaghan, University of Reading
Pond dyes are a cosmetic product for garden ponds and lakes; they inhibit algal growth and improve the overall appearance of the water body. The dyes block red light from entering the water, interrupting the process of photosynthesis and therefore inhibiting the growth of certain aquatic plants such as algae. Although these dyes are not directly toxic to fish and invertebrates, their impact on freshwater communities has yet to be studied. At Reading, we are studying the impact of pond dyes on mosquito colonisation of pond ecosystems and the effect on mosquito community composition. To address these issues we are undertaking mesocosm studies as well as laboratory studies. Here we present data on the impact of a black pond dye on the oviposition of British mosquito species. Females lay eggs in many different types of water body including ponds, shallow lakes and artificial containers. Although British mosquito species vary in their oviposition suitable of conditions, there is a bias towards water and are known to compete with other aquatic invertebrates such as water fleas. We undertook a number of oviposition choice experiments to compare water treated with and without dye at the concentrations specified by the manufacturer. Captive gravid Culex pipiens female mosquitoes (wild caught and laboratory strains) were presented with a choice of containers with or without dye in a field, semi-field (wild caught in a tent) and laboratory conditions. Our preliminary results show that there is a significant difference between the treatments. The black dye is attractive to gravid mosquitoes, encouraging them to lay eggs preferentially in dark waters. Since these mosquitoes are known to be competitors of cladocerans and other macroinvertebrates, the dye is possibly altering the community composition in ponds. Although the pond dye is not exerting a direct chemical stress on the community, it is a potential engine of community change.

TU106
Comparing the impact of pesticide treatment on arthropod communities in different studies using trait based analyses
S. Aldershof, Bioresearch & Evaluation; F.M. Bakker, Mitox Consultants The impact of pesticides on terrestrial arthropod communities prevalent in agro-ecosystems are commonly evaluated with ordination techniques (i.e. Principal Response Curves Analysis, 1). Community responses towards pesticide treatments on different locations can not be compared directly due to the fact that species composition differ in different ecozones. This problem can be overcome by describing communities by an identical set of trait values rather than by taxonomic classification. Poster illustrates a method to compare arthropod community responses in two apple orchards in Northern and Southern Europe. Shifts in functional diversity (2) related to pesticide treatment were compared directly in the two studies by performing Principal Response Curves Analysis on Community Weighted

TU107 Characterization of Qatari Porites (Growth and Photosynthetic Efficiency) Under Dynamic Variables, An Experimental Approach
N.M. Al-Naema, ExxonMobil Research Qatar / Environmental Program; N. Deh, ExxonMobil Research Qatar / Environmental; S. Saeed, ExxonMobil Research Qatar; R. Ben-Hamadou, Qatar University / Department of Biological and Environmental Sciences; M. Al-Ghouti, M. Abu-Deyeh, Qatar University; D. Dupont, ExxonMobil Research Qatar

ABSTRACT Coral ecologies are very essential as they offer a habitat to marine species. Coral reefs are known to be under threat due to stressors to coral health. Samples were collected and cultured in pre-acclimatized lab aquaria. Orthogonal experiments for 2 weeks were conducted under stress stimuli: salinity, light intensity and temperature. Imaging-Pulse Amplitude Modulation Fluorometry (Imaging-PAM) and buoyant weight were utilized to measure photosynthetic performance and growth of the corals. We were able to detect biological responses of the corals to stress stimuli using PAM parameters Maximum Quantum Yield (Fv/Fm), Electronic Transfer Rate (ETR) and Non-Photochemical Quenching (NPQ). OBJECTIVE To establish corals culture under controlled conditions and detect biological and physiological responses to different levels of stress stimuli: salinity, light intensity and temperature using PAM and buoyant weight.

METHODLOGY Coral samples were collected from mother colonies from different localities in Qatar. Acclimatization process started gradually at lab by adding corals into pre-acclimatized lab aquarium. Corals were stressed for 12 days under stress stimuli (separately): salinity, light intensity and temperature. PAM measurements were taken every 3 days. Weight of individual corals was measured at the beginning and the end. RESULTS & DISCUSSION Results revealed that elevated levels of temperature and salinity have statistically significant effect on Symbiodinium photosynthetic activity; while light intensity did not. High level of salinity (50 psu) affected the corals photosynthetic efficiency by causing a drop to a very low range (0.28). As a result, corals weight dropped down too. In contrary, elevated light intensities showed normal range of efficiency and growth rate increased with increasing light levels. Finally, Portites Symbiodinium are highly sensitive to increased temperature up to 38°C where they exhibited complete death. “bleached” and PAM parameters dropped to zero value. CONCLUSION Using high-resolution fluorescence PAM combined with its derived detailed image and gain percentage allowed us to identify the stress that is caused by elevated levels of parameters. High salinities (50 psu) and temperatures (38°C) affect the photosynthetic activities of Portites Symbiodinium. FUTURE WORK PAM assessment to genetic identification tools by correlating species tolerance to different stress stimuli sounds to be very promising to understand coral response mechanism.

TU108 Oscillating population dynamics, nutrient cycling and the subsequent effects on exposure sensitivity to daphnids and algae
D. Eidsvik, Norwegian Institute for Water Research NIVA; L. Nizzetto, NIVA; A. Lillicrap, NIVA / Ecotoxicology and Risk Assessment

The concept of assessing the ecotoxicity of chemicals under realistic environmental situations with increasing levels of complexities has become more frequently recognized and considered to be a holistic risk assessment approach. The direct and indirect effects of chemical contaminants on trophic interactions in a structured ecosystem model (such as for example an oscillating predator-prey dynamic) are not currently part of standard laboratory assays, while they may represent very sensitive “toxicity” endpoints. In order to assess the function of ecosystem dynamics with multiple stressors, we have developed a system including one primary producer (Pseudokirchneriella subcapitata) and a primary consumer (Daphnia magna). These chemostats are relatively self-sufficient, running for multiple generations over several months with oscillating biomass of both algae and daphnids exhibiting boom and bust outcomes in the populations. Whilst it is recognised that increasing the level of complexities may cause challenges, it is envisaged that performing ecotoxicity assessments using such approaches will provide a more environmentally realistic assessment of the effects of chemicals in the environment.

TU109 Assessment of impacts caused by remediation technologies on phytoplanktonic and benthic communities of an urban eutrophic reservoir A.E. Sueitt, A.A. Mozeto, Federal University of Sao Carlos / Chemistry and Environmental Sciences; C. dos Santos, Federal University of Sao Carlos / Environmental Sciences; M. Al-Naema, Norwegian Institute for Water Research NIVA; L. Nizzetto, NIVA; A. Lillicrap, Norwegian Institute for Water Research NIVA / Ecotoxicology and Risk Assessment; D. Eidsvoll, NIVA / Ecotoxicology and Risk Assessment

D. Eidsvoll

TU110 Interactions between chemical stress and dispersal in marine phytoplankton
L. Baert, Ghent University / Bioscience Engineering; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; A. Baert, Ghent University / Bioscience Engineering; C. Baert, Ghent University / Bioscience Engineering; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology

Ghent University

TU111 Technical realisation and validation of an outdoor stream mesocosm L. Dören, MESOCOSM GmbH Institut für Gewässerschutz; P. Janz, Technische Universität München; E.R. Rodríguez, Hochschule RheinMain; K. Ebke, MESOCOSM GmbH, Institut für Gewässerschutz

A new outdoor test system was constructed to study effects on stream organisms.
under realistic conditions. All components of the test system which come into contact with water are made of stainless steel. The model streams are “recirculating” modular systems which can be adapted to the specific requirements of each study (e.g. water level, current velocity,…). Each model stream has a water depth of 0.3-0.5 m, a volume of 1400-2400 L and a water course of about 10 m. The water is driven by paddle wheels with a current velocity up to 0.5 m/s. Since the hydrodynamic factors play a key factor for the distribution of stream mesocosms, they should be taken into account when developing stream mesocosms. Preliminary studies were conducted in order to optimize the flow characteristics. An equal current velocity distribution along the cross section as well as along the water course was realised by the installation of flow conditioners and guide plates. These installations also ensure a high reproducibility between the model streams regarding the flow characteristics. Furthermore, specific sampling techniques to monitor leaf decomposition, phytoplankton, periphyton, macroinvertebrates, emerging insects and drifting organisms were adjusted and validated.

TU112 Does habitat connectivity reduce the amount of stress-tolerance variability needed to preserve ecosystem functions?

J. De Baerd. Laboratory for Environmental Toxicology and Aquatic Ecology; J. Baert. Ghent University / Bioscience Engineering; F. De Laender. Université de Namur ASBL / Laboratory of Environmental Ecosystem Ecology; C. Janssen. University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology GhEnToxLab unit

This study showed that habitat connectivity increased the stability and resilience of the ecosystem. The results of this study can be used to guide the conservation and management of these ecosystems.

TU113 Schemes for further Consideration of Biodiversity issues in Ecological Risk Assessment

E. Chaideftou, Benaki Phytopathological Institute / Department of Pesticides Control Phytopharmacy Laboratory of Toxicological Control of Pesticides

Ecological Risk Assessment (ERA) entails the identification and characterization of Ecological Effects towards the Characterization of Risk from Exposure posed to a specific Environmental Entity. For the Characterization of Ecological Effects to the Entity, the method for stress elicited by the stressor are identified and quantified and the causal relationships are potentially evaluated. For the Determination of Exposure of the Entity, the spatiotemporal distribution of a stressor and the occurrence or contact of stressor with the ecological components is predicted or measured. Specific Protection Goals are determined in ERA after defining the Entity under protection. Each Entity is expressed by a specific Environmental Component or by an entity. Among the entities which increased migration reduced the impact of atrazine on ecosystem performance (p<0.0001). Analysis of a stochastic Lotka-Volterra model indicated a trade-off between migration rate and community stress tolerance variability to buffer ecosystem functions: better-connected communities need a smaller degree of interspecific stress variability (i.e. a steeper species sensitivity distribution) to buffer ecosystem functions in the same way as less-connected communities. Both empirical and theoretical evidence suggest that landscape features can be important for future risk assessment.

TU115 Anthropogenic activities drive the microbial community and their functions in the river bed sediment

X. Zhang. University of Waterloo / Department of Biology; Q. Gu. Zhejiang Environmental Monitoring Center; X. Long. Chinese Academy of Sciences / Institute of Urban Environment; Z. Li. Fudan University / The Institute of Biodiversity Science; D. Liu. D. Ye. C. He. X. Liu. Shanghai University / School of Environmental and Chemical Engineering; K.K. Vannanen. University of Eastern Finland / Biology; X. Chen. Shanghai University / School of Environmental and Chemical Engineering

Understanding the dynamics of the structure and function of the sediment microbial community across freshwater ecosystems will help to predict how these ecosystems will change in response to anthropogenic activities. Materials and methods We compared the community structure and abundance of C/N transformation-related functional micro-organisms along two rivers in South-Eastern China that flow through three typical functional zones corresponding to agricultural, residential and industrial zones. Results and discussion Illumina sequencing of 16S rRNA amplicons revealed that the sediments are dominated by Proteobacteria (41.4-54.4 %) at all of the tested sites and demonstrated notable differences among the communities from different functional zones. The ratio of AO A:AOB amoA gene copy numbers in the agricultural zone was below 1.0, whereas those in both the industrial and residential zones were above 1.0 (1.10-5.34). Non-metric multidimensional scaling (NMDS) analysis showed that the microbial communities in the agricultural zone were mainly correlated with volatile phenol, and NH₃, CO₂, and CO₂ changed significantly higher than in the residential and agricultural zones. These findings indicate that the fluctuations of the microbial community in river sediment are impacted by different human activities, and particular functional groups, such as nitrogen fixers (nifH), nitritifiers (AOB), and methanotrophs (pmoA), are sensitive to environmental changes.

TU116 Use of mesocosm study data in current and future risk assessments - a case study

J. Postma. R. Keijzers, Ecofide; A.D. Redman, Exxon Mobil Biomedical Sciences; C.V. Eadsworth, Shell International; S. Linington, BP Castrol Technology Centre; S. Comber, Plymouth University / Environmental Science; M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences TES Division; K. den Haan, CONCAWE / Petroleum Products Safety

The results of this study can be used to guide the conservation and management of these ecosystems. Significant decrease of the risk in the agricultural zone could be achieved by the introduction of new management practices such as optimized use of fertilizers and pesticides, and the implementation of best environmental practices. In estimating PNEC-values or the derivation of environmental quality standards (EQS), data from mesocosms can be used to argue that lower safety factors might be applied. The decision on the size of such an application factor (AF) is made on a case-by-case basis. A literature review was conducted to provide insight in the present use of available mesocosm data in risk assessments and in several technical aspects in relation to study design. The main conclusions were: A large amount of information on mesocosm studies is available and there is not just one approach. All aspects of test design should be discussed and optimised in the light of the specific research objective. Available literature reviews on a limited amount of data indicate that the SSD approach, in which single-species studies are used to calculate a HDI, is normally not applied in risk assessments of hazardous materials. Replicability and repeatability of physical, chemical but especially biological parameters should be assessed as part of the test design. An experimental design encompassing more than 4 concentrations and at least two replicates is suggested in order to obtain sufficient resolution (Mühlenberg et al., 2001). Options to reduce variability can be ’n’-to locate mesocosms near to the source system and allow communities to establish naturally- to control the settling of sediment and grain size distributions ‘n’-to allow continuous recolonisation during the experiments. The size of mesocosms itself is not one of the driving forces for the biological complexity of stream mesocosms. Nevertheless, several parameters are impacted by size such as mixing zone, flow-rate and sampling zones. These need to be carefully considered when setting up mesocosm experiments. Flow-through mesocosms, with a continuous inflow from upstream, can be highly replicable and can be considered realistic. The realism of the biological community tested in a mesocosm should be assessed as one of the elements in a weight of evidence approach in support of a low AF. Validating the ecological realism of mesocosms using “dimensional analysis” should be considered. Statistical considerations form
an essential part of both study design and interpretation. The use of multivariate analysis and especially the Principal Response Curve method has become state-of-the-art for interpreting mesocosm data.

TU117

A new outdoor test system with stream mesocosms

P. Jata, Technische Universität München; L. Dönk, K. Elke, MESOCOSM, P. Günthard, Institut für Gewässerschutz

Lentic (static) aquatic mesocosms are most frequently used to refine aquatic risk assessments of pesticides when a potential risk is indicated by lower tier studies (e.g. laboratory single species tests). However, small lotic water bodies (small streams and ditches) in agricultural areas are often the most exposed water bodies. Lotic water bodies differ strongly from lentic water bodies in terms of their abiotic environmental factors and their biocenoses. Thus, the extrapolation of results from lentic mesocosms studies to small lotic water bodies is subject to many uncertainties. These uncertainties can be reduced if lotic mesocosms are used instead. A new outdoor test system with artificial streams was designed, with particular focus on macroinvertebrates including the EPT taxa (Ephemeroptera, Plecoptera, Trichoptera) and gammarids. In a pilot study a representative community containing the important taxonomical groups, trophic groups and ecological traits (particularly life-cycle characteristics related to vulnerability as generation time and dispersal ability) typical for communities in small streams could be established. Further it was shown that variations between the model streams were very low for physico-chemical parameter, leaf decomposition, phytoplankton and periphyton throughout the entire study period of 8 weeks. Minimum detectable differences (MDDs) were calculated to indicate the statistical power of the mesocosms. The MDD defines the difference between the means of the treatment and the control that must exist in order to conclude that there is a significant difference. According to the MDD calculations the variability between the model streams was sufficiently low in order to enable a statistical evaluation for more than the (required by FSV) 8 weeks. The differences of the target parameters (including the EPT taxa data) between the test system meets all criteria for mesocosms according to the FSV guidance document on tiered risk assessment for edge-of-field surface waters and it is considered as a very powerful tool to study the effects of pesticides on stream organisms under realistic conditions.

Latest advances in passive sampling and dosing (P)

TU118

POCIS for pesticide monitoring in groundwaters: from "low flow" to "high flow" calibration in situ monitoring

a. togoa, C. Berho, BRGM / Laboratory Division, S. Robert, Suez Environment / CIRSEE; A. Bruchet, CIRSEE Suez Environment

Since few decades, polar organic chemical integrative sampler (POCIS) has been successfully applied to the sampling of a wide range of polar organic contaminants including pesticides. Passive sampling offer time integrated sampling that compensates for fluctuations in concentrations and lower detection limits compared to standard water sampling. Applications have been implemented in surface water or wastewaters after obtaining sampling rate (Rs) by laboratory calibrations. POCIS sampling rates are affected by environmental factors such as water flow rates, that is particularly significant for groundwaters, characterized by low water flow conditions. To describe the relation between water flow and POCIS accumulation, an experimental system has been implemented to obtain sampling rates for POCIS on representative groundwater hydraulic conditions of alluvial aquifers. In parallel, monitoring campaigns have been undertaken during one year to obtain in situ sampling rate values. This works compares this two approaches with classic in lab results, to determine impact of flow on POCIS accumulation in groundwater context. By this way, lab calibration that is easiest to implement, could be used and corrected to better fit with environmental condition, allowing the use of passive sampler for a semi-quantitative approach.

TU119

Passive samplers as cost-effective WFD screening devices

J.M. Ronan, Marine Institute / Chemical Sciences; L. Jones, Dublin City University / Chemical Sciences; B. McHugh, E. McGovern, Marine Institute; f. Regan, Dublin City University / Chemical Sciences

The primary purpose of the WFD is to establish a framework for the protection of all the waters throughout the EU territory. Considering appropriate water and biota sampling to meet WFD monitoring requirements is both costly and challenging, given the analytical difficulties posed by low environmental quality standard (EQS) values, the availability of suitable and sufficient numbers of biota (for EQSmono), and the volume and frequency of sampling required. Passive sampling (PS) techniques are rapidly developing as very cost-effective state-of-the-art pragmatic tools to identify and measure ultra-trace micro-pollutants in water as improved compound detectability and sensitivity may often be obtained relative to more "traditional" spot water sampling and analysis techniques. It is proposed that PS derived dissolved pollutant data in tandem with appropriate modelling techniques may be suitable for the derivation of individual precautionary principle based parameter “passive sampling EQS equivalent” (EQSmono) screening/threshold values. When used within a tiered monitoring framework PS screening results would then be assessed against the derived thresholds whereupon exceedance may initiate further more focused sampling. It is thus suggested that this combination of PS thresholds within a tiered approach provides a cost effective option for surveillance monitoring to highlight possible compounds and locations of environmental concern.

TU120

Applying silicone-based passive samplers in water monitoring

B. Becker, Department of Qualitative Hydrology; A. Duffek, Federal Environment Agency; P. Lepom, Laboratory for Water Analysis; A. Paschke, Helmholtz Centre for Environmental Research (UFZ); C. Möhlenkamp, Federal Institute of Hydrology; E. Claas, Federal Institute of Hydrology; B. Göt; S. Scharfer, Federal Institute of Hydrology / Department of Qualitative Hydrology

Quantifying hydrophobic organic chemicals (HOCs) in water is challenging due to the low concentrations which need be measured and the insufficient detection limits which are often achieved when taking 1 L spot samples. Passive samplers can accumulate HOCs while being exposed in the environment enabling not only low detection limits but also the measurement of time-weighted average concentrations (cTWA). In this study, silicone-based passive samplers were applied to monitor water quality in rivers. Silicone rubber sheets were exposed in situ at five sampling sites in different rivers in Germany (Elbe, Rhine, Danube, Saale and Saar). Three sampling campaigns were conducted from May until October 2014 with an exposure time of five weeks each. Target analytes were PAHs, PCBs as well as DDT and its metabolites. Analyte-specific sampling rates (Rs) were determined by in situ calibration with ten performance reference compounds (PRCs). The remaining amounts of PRCs after the exposure period were measured. Rs were calculated by non-linear regression of the PRC fraction, which remained in the samplers and analyte-specific silicone-water partition coefficients according to Rusina et al. (2010). Abiotic factors such as water temperature and flow rate in the rivers were measured to study their effect on Rs of target analytes. Next, cTWA of the target analytes were calculated at each sampling site based on the in situ calibration with PRCs. Results from the first sampling campaign in 2014 showed different contamination patterns for the five sampling sites. The highest PCB cTWA of 110 pg/L was found for CB 138 in the Elbe river. PAH concentrations were in the pg/L range up to a few ng/L. PAH concentrations ranged from 2 pg/L (indeno[1,2,3-c,d]pyrene in the Danube to 3.2 ng/L for fluoranthene in the river Saar. The PAH patterns varied between the different rivers with a higher fraction of the more hydrophilic PAHs seen in the rivers Saar, Danube and Rhine. As expected highest levels of DDT and its metabolites were measured in the Elbe river with up to 600 pg/L for p,p'-DDD. Detection limits of target analytes were below 1 pg/L depending on Rs. This comparison will compare the cTWA obtained for the different sampling sites and sampling campaigns. Also the potential and limitations of implementing passive samplers in routine monitoring campaigns, e.g. for monitoring under the European Water Framework Directive, will be discussed.

TU121

Targeted screening of emerging pollutants in Czech rivers by passive sampling

V. Kodes, Czech Hydrometeorological Institute / Section of water quality; R. Grabic, University of South Bohemia in CB / Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocnoses

A new outdoor test system with stream mesocosms was developed for the purpose of protecting all the waters throughout the EU territory. Concentrating appropriate water and biota sampling to meet WFD monitoring requirements is both costly and challenging, given the analytical difficulties posed by low environmental quality standard (EQS) values, the availability of suitable and sufficient numbers of biota (for EQSmono), and the volume and frequency of sampling required. Passive sampling (PS) techniques are rapidly developing as very cost-effective tools to identify and measure ultra-trace micro-pollutants in water as improved compound detectability and sensitivity may often be obtained relative to more “traditional” spot water sampling and analysis techniques. It is proposed that PS derived dissolved pollutant data in tandem with appropriate modelling techniques may be suitable for the derivation of individual precautionary principle based parameter “passive sampling EQS equivalent” (EQSmono) screening/threshold values. When used within a tiered monitoring framework PS screening results would then be assessed against the derived thresholds whereupon exceedance may initiate further more focused sampling. It is thus suggested that this combination of PS thresholds within a tiered approach provides a cost effective option for surveillance monitoring to highlight possible compounds and locations of environmental concern.
Medicina Clinica e Molecole; E. Cocco, M. Atzori, Universita di Cagliari / Dipartimento di Sanita Pubblica Medicina Clinica e Molecole

This work presents the results of two years monitoring of organics and trace metals in seawater with passive sampling techniques in the area around the wreck of the Costa Concordia cruise ship, which sank off the Mediterranean island of Giglio (Italy) on January 2012. The aim was to monitor bioavailable contaminants released by the wreck and/or produced by the removal yard (Parbuckling project). Sampling was carried out at three sites from May 2012 to September 2014, one month after the removal of the wreck. Two sites were fixed in the vicinity of the wreck, at the bow and at the aft of the ship; the third one was located in an unexposed beach of the island. Nine sampling campaigns were carried out. In each station a casiter containing semipermeable membrane devices (SPMDs) and polar organic chemical indicator sampler (POCIS) was deployed at 10 m depth, fixed at a buoy by scuba divers. Furthermore, at each casiter, 9 diffusive thinss in films (DGTs) devices were attached. DGTs with three different resins have been used: Chelex-100 for Cd, Cr, Cu, Ni, and Pb; Fe-oxide for V; Spheron-thiol for Hg. Deployment time for all samplers ranged from 5 to 7 weeks. Repeatability was checked by the exposure of triplicate samplers. SPMDs and POCIS were extracted and analyzed for target compounds and partitioning. Results show higher concentrations of PAHs in the station near the bow of the ship, all along the monitoring period. A significant correlation with V measured by DGTs was pointed out, indicating a contamination by oil, more likely as a result of the yard activities. This work is part of a larger monitoring plan financially supported by the Italian Civil Protection.

TU123 Equilibrium sampling of hydrophobic organic chemicals in sediments: challenges and new approaches

S. Schaefer, Federal Institute of Hydrology / Department of Qualitative Hydrology; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; B. Becker, Department of Qualitative Hydrology, C. Möhlenkamp, Federal Institute of Hydrology; E. Clauss, Federal Institute of Hydrology Big; F. Smedes, DELTARES / BGS

The exposure risk of contaminated sediments is traditionally assessed by exhaustive extraction methods that measure total concentrations (ca) including bound and freely dissolved contaminants. However, freely dissolved concentrations (cf) of hydrophobic organic chemicals (HOCs) are considered to be the effective concentrations for bioavailability and the uptake and partitioning, and they can be measured by equilibrium sampling. We have thus applied glass jars with multiple coating thicknesses for equilibrium sampling of HOCs in sediment samples from various sites in different German rivers. The coated glass jars were very convenient for routine monitoring campaigns since (1) equilibrium times are minimized by the very thin coatings, (2) the equilibration is done in the laboratory and (3) equilibrium sampling is avoided. A linear least square regression of analyte concentrations in polymer as a function of silicone mass using a first order kinetic model. Finally, the model was validated by the exposure of triplicate samplers. SPMDs and POCIS were extracted and analyzed for target compounds and partitioning. Results show higher concentrations of PAHs in the station near the bow of the ship, all along the monitoring period. A significant correlation with V measured by DGTs was pointed out, indicating a contamination by oil, more likely as a result of the yard activities. This work is part of a larger monitoring plan financially supported by the Italian Civil Protection.

TU125 Remediation of dioxin-contaminated marine sediments using thin-layer capping with activated carbon and other sorbents: evaluation of bioavailability assessment techniques

J. Gunnarsson, A. Gustafsson, Stockholm University / Department of Ecology and Environmental Plant Sciences DEEP, S. Josefsson, Swedish University of Agricultural Sciences / Dept of Aquatic Sciences and Assessment; I.J. Allan, Norwegian Institute for Vannforskning; G. Cornelissen, NGI, M. Schaaning, Norwegian Institute for Water Research

Remediation of contaminated sediments in situ using thin-layer capping with carbonaceous materials such as activated carbon (AC) has proven successful in reducing the bioavailability and say to deposed passive samplers. Studies in the use of organic compounds, e.g. PCBs, PAHs and dioxins, by several orders of magnitude. In two joint projects called CARBOCAP and OPTICAP, Swedish and Norwegian researchers have evaluated various capping materials for thin-layer capping in situ, using both mesocosms and large-scale field experiments. In the present study we collected sediment from a dioxin-contaminated area in the Grenlandfjord, Norway. The sediment was placed in boxes and a thin-layer capping (1-5 cm) of either clay or crushed limestone was added to the surface. The caps were amended with two types of carbonaceous sorbents activated carbon (AC) or Kraft Lignin (KL). Two test organisms, a surface gastropod (Nassarius nitidus) and a deep burrowing polychaete (Nereis spp.) were added to the boxes for bioaccumulation assays. Passive samplers (SPMDs) were placed in the water column and the boxes were held at constant ambient temperature in a flow-through mode for 99 days. At the end of the experiment passive samplers, animals and sediment surface samples (0-3 cm) were collected for chemical analyses of dibenzo-p-dioxins and dibenzofurans, hexachlorobenzene, and octachlorostyrene. Bioavailability analyses of these compounds were conducted using synthetic digestive fluid extraction (DFE) or direct digestive fluid extraction (LDPE). Measurement of active sorbents (AC or KL) led to a significant reduction in bioaccumulation by the two test species. AC was more efficient than KL as sorbent. A 90% reduction in bioaccumulation was obtained with 3 cm caps with 3.3% AC. DFE and LDPE measurements both corresponded well with the bioaccumulation results. The addition of a 3 cm cap amended with AC measured by DFE resulted in a 93% reduction dioxins/furans-bioavailability, compared to a 91% reduction in the thagastropod and a 75% in the polychaete. Our results are promising in terms of developing faster screening tools for assessing toxicity risks to bionia from contaminated sediments, and to evaluate the efficiency of various capping materials, as these bioavailability methods are more rapid (days instead of months) and more cost-efficient compared to traditional bioaccumulation assays using live organisms.

TU126 The Use of Passive Samplers to support the Short-term Performance Assessment of In Situ Remedial Treatment at Two Contaminated Sediment Sites

V.J. Kirtay, SPAWARSYSNCD SEN DIEGO / Energy and Environmental Sustainability; J.M. Conder, Geosyntec Consultants; M.M. Grover, ENVIRON International Corporation; G.H. Rosen, SPAWAR Systems Center / Energy and Environmental Sustainability; B. Chadwick, SPAWAR Systems Center San Diego / Energy and Environmental Sciences

Passive samplers are being used as part of a weight-of-evidence approach to evaluate the effectiveness of in situ remediation for reducing contaminant availability at two different contaminated sediment sites. At the first contaminated sediment site, two to three inches of AquaGate+PACTM (a composite aggregate coated with activated carbon) was placed on a 0.5-cm target area to reduce polychlorinated biphenyl (PCB) bioavailability in sediment containing PCBs underneath and adjacent to an active pie. As part of the monitoring program, concentrations of freely dissolved PCB congeners in surface sediment pore water have been measured via in situ solid phase microextraction (SPME) at ten stations which received amendment both prior to and at 10- and 21-months following amendment placement. For each survey, passive samplers were constructed using twelve 12.5-cm pieces of fiber with 10-µm
thickness polydimethylsiloxane (PDMS) coating and a 210-μm silica core diameter. The fiber pieces were placed in a 110-μm stainless steel mesh envelope. Within two weeks of deployment the passive samplers were exposed to a solution containing PCB Performance Reference Compounds and stored at 4°C. On the day of deployment, each sampler was attached to a SEA Ring bioaccumulation chamber and deployed in situ for 14 days. Upon retrieval, each sampler was wrapped in foil and placed on ice for shipment to the laboratory for processing and analysis. At the second contaminations were tested, a similar approach is being used to evaluate the effectiveness of a thin layer cap for reducing the availability of DDT and its daughter products (DDX) to the benthic community. At this site, a six-inch layer of sand was placed over a 10-acre site. Concentrations of DDX were measured in sediment pore water in core samples collected from five on cap stations and two reference stations both prior to and at 12 months post cap installation. For this site, a similar approach was used to construct the passive samplers. However, because of the interest in evaluating the extent to which top-down versus bottom-up processes control the potential for recontamination of the cap surface, the core samples were sectioned and a passive sampler was added to each section and tumbled ex situ for two weeks prior to processing and analysis. Results from the pre-placement monitoring and post-treatment monitoring will be presented in terms of the effectiveness of the passive sampler to evaluate predictions in contaminant availability.

TU127 Using equilibrium passive dosing to maintain stable exposure concentrations of triclosan in a 6-week toxicity test
A. Sobek, Applied Environmental Science ITM; A. Ribbenstedt, Stockholm University; L. Mustajärvi, Stockholm University / Applied Environmental Science ITM; M. Breitholtz, Inst for tillämpad miljövetenskap / Department of Applied Environmental Science ITM; P. Mayer, Technical University of Denmark / Department of Environmental Engineering Aquatic animals are constantly exposed to hydrophobic organic contaminants. Although these chemicals may be present at low concentrations, they can still cause negative long-term effects on organisms. In chemicals risk assessment, the majority of the ecotoxicological data is based on acute toxicity tests. Yet, the European Commission’s criteria for chemicals’ risk assessments aim at protecting higher levels in the environment. To achieve a protection of populations and ecosystems, reliable long-term ecotoxicological tests are needed. In this study, we used equilibrium passive dosing to maintain stable exposure concentrations of triclosan (log Kow, 4.8) in a 6-week multigeneration test with the benthic copepod Nitocra spinipes. The tests were performed in 10 mL vials casted with 1000 mg of silicone (DC 1 2577). Based on a previous pilot study, three triclosan concentrations were set for the test: 0.5, 1, and 2 mg L-1 as well as a control (no triclosan). At test beginning, each vial contained 12 individuals consisting of 3 individuals from four different life stages. The test includes feeding with phytoplankton three times a week, which can lead to declining freely dissolved triclosan concentrations. In the present study this was buffered by passive dosing. Water was exchanged every 7th day by transferring the animals from the spend solution into a pre-equilibrated passive dosing glass. During the first two weeks, the copepods grew exponentially and can reach numbers of several hundred individuals in each vial. The increasing biomass of the test organisms can again lead to declining exposure concentrations, but such loss was buffered by passive dosing. Quality assurance tests showed that a) the loading and passive dosing procedures resulted in exposure concentrations with little variation (RSD 1-7 %), b) using two batches of passive dosing vials helped to maintain stable exposure concentrations and c) the concentration of triclosan could be maintained throughout the whole 6-week test period. This study demonstrates that passive dosing offers a way forward to generate reliable and relevant toxicity data also from long-term studies with hydrophobic contaminants. The results on long-term toxicity of triclosan on Nitocra will be evaluated and discussed in relation to existing toxicity data from literature.

TU128 Effect-based monitoring of Danube river using mobile passive sampling approach
L. Mustajärvi, Masaryk University / RECETOX; B. Vrana, Masaryk University; Faculty of Science, RECETOX / RECETOX Research Centre for Toxic Compounds in the Environment; F. Smedes, DELTARES / BGS; T. Rusina, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; K. Okonski, Masaryk University Faculty of Science / RECETOX Research Centre for Toxic Compounds in the Environment; B.I. Escher, Helmholtz Centre for Environmental Research UFZ / UFZ Cell Toxicology; M. Macova, The University of Queensland / National Research Centre for Environmental Toxicology Entox; P.A. Neale, Griffith University / Smart Water Research Centre; S. Ait-Aissa, INERIS / Ecotoxicology Unit; N. Creuset, INERIS; K. Hilscherova, Masaryk University, Faculty of Science, RECETOX / RECETOX Research Centre for Toxic Compounds in the Environment.
Surface waters are often polluted by complex mixtures of pollutants whose toxic properties are relatively difficult to describe. Effect-based monitoring with a battery of bioassays represents an efficient approach for toxicological profiling and identification of samples of major interest through providing data of the toxic potencies and mode of action of the extracted mixtures that cannot be evaluated by chemical analysis alone. In our work we used an “active” passive sampling system (APS) for temporally and spatially integrative sampling using silicone rubber and EMPORE disc based passive samplers, which efficiently preconcentrate a wide spectrum of polar and non-polar organic pollutants from the water column along a defined river stretch. This concept was employed during joint Danube Survey 3 in summer 2013. Eight Danube river stretches were sampled to cover spatial distribution of the pollutants characterized through a set of bioassays investigating presence of contaminants with severe and important specific toxic modes of action. Presence of contaminants with potentials for causing endocrine disruption (anti/estrogenic anti/androgenic), dioxin-like activities, potential to elicit adaptive stress responses associated with oxidative stress, p53-mediated apoptotic response and NF-kB-associated inflammatory response were characterized in samples from different river stretches. This wide range of assessed endpoints serves as a toxicological profiling tool to describe risks associated with fresh water pollutant mixtures. The obtained results also serve for spatial characterization of pollution gradients along the river and selecting hotspots for further studies. This research was supported by SOLUTIONS Project from the European Union Seventh Framework Programme (FP7-ENV-2013-two-stage Collaborative project) under grant agreement 603437.

TU129 Time Integrative Passive sampling combined with Toxicity Profiling (TIPTOP): an effect based strategy for cost-effective chemical water quality assessment
T. Hamers, VU University Amsterdam, Institute for Environmental Studies (IVM) / Institute for Environmental Studies; F. Smedes, DELTARES / BGS; j. de Weert, DELTARES; L. Posthuma, D. Zwart, RIVM / Centre for Sustainability Environment and Health; P. Leonards, VU University, Institute for Environmental Studies / Institute for Environmental Studies IVM Within the European Water Framework Directive (WFD) chemical water quality is assessed by comparing concentrations of specific and individual priority pollutant to their Environmental Quality Standard (EQS). This approach ignores not only non-analyzed (known or unknown) compounds that may contribute to the toxic burden of the water, but also mixture effects. In addition, it is based on a time-point grab sample that may miss incidental peak concentrations. We hypothesize that a combined approach of time-integrative passive sampling followed by toxicity profiling is not only a toxicologically and ecologically more relevant and more protective alternative for chemical water quality assessment, but also a more cost-effective alternative. For this purpose a demonstration study is designed at well-defined WFD sampling sites in the Dutch delta. Time-integrative sampling is performed using partitioning-based and adsorption-based passive samplers. Toxicity profiling is performed using a test battery consisting of in vitro and in vivo bioassays. Given the different mechanisms of action covered by the in vitro bioassays and the different trophic levels covered by the in vivo bioassays, the applied battery can be regarded as “safety net”, signalling the mixture effect of toxic compounds at low concentrations. Ultimately, different approaches will be used to determine the surface water chemical status, i.e. (1) by the classical WFD assessment based on priority compound analyses, (2) by calculating the observed chemical profile into a toxicity profile and compare that to the actually observed toxicity profile, and (3) by a toxicological or ecological interpretation of the observed toxicity profile itself. The toxicological interpretation is based on a comparison to “worst case” toxicity profiles from waste-water treatment plant effluent or to trigger values for bioassays. The ecological interpretation is based on a comparison between predicted ecological effects and the actual ecological status and on the assessment of a margin of exposure in the field situation to expected ecological effects. The combined approach of passive sampling and toxicity profiling will be evaluated according to SWOT principles, using experiences from TIPTOP, previous studies from the partner institutes, and similar studies published in open (grey and peer-reviewed) literature. The outcome should ultimately lead to a proposal for a cost-effective approach for chemical water quality assessment.

TU130 Transferring the chemical activities of chemical mixtures from sediment to toxicity tests using silicone-based passive sampling and dosing
C. Mattavelli, Stockholm University / Applied Environmental Science ITM; A. Jahnke, Stockholm University / Department of Cell Toxicology; A. Eriksson, ITM Stockholm University / Applied Environmental Science ITM; A. Sobek, Applied Environmental Science ITM
This study aims at validating an approach for transferring mixtures of the bioavailable fraction of hydrophobic organic contaminants (HOCs) from sediment to toxicity testing, using a silicone-based passive sampling approach (consists of five steps): 1) partition-based sampling of HOCs into a sampling polymer; 2) solvent extraction of HOCs from the sampling polymer; 3) transfer of the HOC extract into a dosing polymer; 4) equilibration of the HOCs in the dosing polymer with the test medium; and 5) in vivo toxicity test, with algae. The freely dissolved fraction of HOCs in sediment was sampled by silicone-coated glass jars (e.g. Reichenberg et al. 2008, Jahnke et al. 2012), a method which has been shown to be successfully used for sediment and soil samples. Each jar contained approximately 180 g wet sediment. The silicone thickness in the jars was modified to 30 μm (0.3 g) to increase the sampling capacity. After three weeks of sampling, established as sufficient time to reach equilibrium by using different coating thicknesses, the
HOCS were extracted with acetone:n-hexane (1:1, v/v), and transferred to the dosing polymer (500 mg) cast in 10 mL vials (e.g. Smith et al. 2010). The loading of the dosing polymer with the HOC extract retrieved from sediment is a critical step, as it must have high precision, be quantitative and avoid swelling of the silicone. At the same time the extract, containing the complex mixture of hydrophobic substances extracted from the sediment, does not dissolve in e.g. methanol which is otherwise required for loading (e.g. Smith et al. 2010). Furthermore, the loading step was evaluated using mixtures of PCBs and PAHs at known concentrations. Furthermore, the whole sampling and dosing procedure was validated by tests with spiked sediment, using triclosan as test compound. The toxicity of increasing concentrations of the mixture of chemicals transferred from sediment will be assessed with respect to phytoplankton cell viability and growth rate, A. maxima, A. marina, E. huxleyi, M.徼thine, Sci. Techn. 2012, 46, 10114-10122. Reichenberg, F., Smedes, F., Jonsson, J.A.; Mayer, P. Chem. Cent. J. 2008, 2, 8, Smith, S.E.C.; Dom, D.; Blust, R., Mayer, P. Aquat Toxicol. 2010, 98, 15-24.

TU131
Comparison of passive and standard dosing of polycyclic aromatic hydrocarbons to the marine algae Phaeodactylum tricornutum
M. Belzunce Segar, G. Witt
TU132
The integration of passive samplers and bioassays to evaluate the water quality of an estuary affected by an effluent plume: case study in the Oiartzun estuary (southeastern Bay of Biscay)
M. Belzunce Segar, Azti-Tecnalia / Marine Research; N. Montero, Azti-Tecnalia; J.A. Laborde, E. Monteyne, C. Schulte, Chem. Cent. J. 2016, 10, 2227-2235. Belzunce Segar, M.; Menchaca, Marine Environment Department; J. Franco, Azti-Tecnalia; The Water Framework Directive (WFD; 2000/60/EC) aims to achieve a ‘good ecological and chemical status’ for all European water bodies by 2015. However, it focuses on the biological elements of the system; therefore, it would be convenient to measure the contaminant concentration that could be more easily related to ecological effects. The objective of this work is to evaluate the applicability of techniques based on the freely dissolved concentrations to characterize the impact of an effluent plume. In order to determine the effect of environmental variables in the speciation of metals and its associated toxicity, sampling campaigns were performed in dry and wet conditions. The Oiartzun estuary (southeastern Bay of Biscay) was selected, as it has been historically contaminated by the surrounding industries and shipyard activities. Twelve stations were chosen along the estuary and water contamination was assessed by means of DGTs. Also, at the outermost stations, located inside the harbour domain, in situ sea-urchin bioassays (48 h) were performed and water samples were collected during a tidal cycle. In the laboratory, the toxicity of composite water samples collected during the tidal cycle was evaluated by the sea-urchin bioassay and Toxicity Identification and Evaluation procedures (TIEs) were applied to identify the chemicals responsible of the observed toxicity. TIEs consist on the physical/chemical manipulation of the samples to reduce the bioavailability of specific contaminants and to establish cause-effect relationships. Different metal distribution patterns were observed in dry and wet conditions by means of DGTs. In general, a decrease in metal concentrations from the mouth to the internal part of the estuary was observed. Moreover, a pollution gradient was observed from the first part of the harbour domain to the mouth of the estuary, as demonstrated by the improvement of sea-urchin larvae development in laboratory and in situ bioassays. Additionally, metals were identified as the contaminants responsible of the observed toxicity by means of TIEs. On basis of our results, the application of techniques based on contaminants labile concentration seems promising for the assessment of the impact of effluents.
Key words: estuary, DGT, in situ bioassays.

TU133
Analysis and ecotoxicological investigations on poorly soluble cosmetic compounds - Project ECOSEmenC
E. Stambou, RWTH Aachen University / Institute for Environmental Research Biology; K. Rettinger, IKW Industrieverband Koerperpflege; J. Steber, German Cosmetic Toiletry, Perfumery and Detergent Association; A. Coors, ECT Oekotoxikologie GmbH; C. Schulte, Umweltbundesamt / Chemicals; H. Hollert, A. Schaeffer, RWTH Aachen University / Institute for Environmental Research Biology includes a broad range of highly hydrophobic compounds that are poorly soluble in water, i.e. far below their EC50 in an acute test like the Brine Shrimp Assay and the typical ‘rinse off’ application, substantial amounts of these poorly soluble chemicals end up in waste-water treatment plants and may subsequently enter aquatic systems with the effluent. Additionally the highly hydrophobic properties of the substances result in extensive adsorption to surfaces like test vessels and organisms. Thus, difficulties in maintaining constant test concentrations occur in standard acute and chronic ecotoxicity tests. This can overcome these problems by the continual partitioning of HOCS from a dominating reservoir loaded in a biologically inert polymer such as silicone. This procedure provides defined and constant freely dissolved concentrations and eliminates spiking with cosolvents. Passive dosing using silicone O-rings as donor and PAHs as test substances (Fluoranthene, Naphthalene, Phenanthrene, Acenaphthene, Fluoranthene, Benzo[a]pyrene, Anthracene and Pyrene) were applied in the marine algal growth inhibition test with Phaeodactylum tricornutum (based on ISO EN 10253) in 24-well microtiter plates. The O-rings were loaded by partitioning from methanol solutions or suspensions of the respective PAHs (1), and these loaded O-rings were added to the wells in test media before the beginning of the test. Agitation of the plates was used to speed up the release from the O-rings. The toxicity of the individual PAHs was investigated at controlled concentrations up to their aqueous solubility in artificial seawater. The concentration-dependent growth inhibition of Phaeodactylum tricornutum was then compared for passive dosing and standard dosing according to the standard marine algae test procedure on microtiter plates. A comparison of the EC50 values of passive dosing vs. EC50 values of standard dosing showed an underestimation of the effects when using nominal standard dosing probably due to sorption, evaporation and limiting dissolution kinetics. Furthermore, passive dosing concentration-response curves were more reproducible and shifted towards lower concentrations. Results show that the response is clearly not only dependent on the potency of the compounds, but also on its supply, sorption and consumption during the assay. Passive dosing is a practical and ecologically more realistic approach than standard high throughput screening approaches for solid substances). First acute and chronic ecotoxicological tests with organisms of different trophic levels as proposed by OECD guidelines (algae, daphnids and fish-eggs) have been performed to verify the ‘poorly soluble approach’ according to the ETNCaP hypothesis.

TU134
Comparison of organic contaminants to marine phytoplankton dynamics: an experimental approach
G. Everaert, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; F. De Laeener, UNIVERSITÉ DE NAMUR / Laboratory of Environmental Ecosystem Ecology; M. Claesens, DuPont de Nemours; J. Baert, Ghent University / Bioscience Engineering; E. Monteyne, Royal Belgian Institute of Natural Sciences MUMM / Management Unit of the North Sea Mathematical PLATFORM; G. Witt, HAW Hamburg / Department of Environmental Engineering; N.C. Niehus, Hamburg University of Applied Sciences (HAW) / Department of Environmental Engineering; K. Konopka, Hamburg University of Applied Sciences HAW / Environmental Engineering; P. Mayer, Technical University of Denmark / Department of Environment Engineering; C. Floeter, HAW Hamburg / Department of Environmental Engineering Testing hydrophobic organic compounds (HOCS), like polycyclic aromatic hydrocarbons (PAHs), in aquatic toxicity tests is difficult due to compound losses through volatilization, sorption to the test vessel and culture medium constituents. This results in poorly defined exposure, the bioavailable concentration is reduced and concentration-effect-relationships might be underestimated. Passive dosing can overcome these problems by continual partitioning of HOCS from a dominating reservoir loaded in a biologically inert polymer such as silicone. This procedure provides defined and constant freely dissolved concentrations and eliminates spiking with cosolvents. Passive dosing using silicone O-rings as donor and PAHs as test substances (fluoranthene, naphthalene, phenanthrene, acenaphthene, fluorene, benz[a]pyrene, anthracene and pyrene) were applied in the marine algal growth inhibition test with Phaeodactylum tricornutum (based on ISO EN 10253) in 24-well microtiter plates. The O-rings were loaded by partitioning from methanol solutions or suspensions of the respective PAHs (1), and these loaded O-rings were added to the wells in test media before the beginning of the test. Agitation of the plates was used to speed up the release from the O-rings. The toxicity of the individual PAHs was investigated at controlled concentrations up to their aqueous solubility in artificial seawater. The concentration-dependent growth inhibition of Phaeodactylum tricornutum was then compared for passive dosing and standard dosing according to the standard marine algae test procedure on microtiter plates. A comparison of the EC50 values of passive dosing vs. EC50 values of standard dosing showed an underestimation of the effects when using nominal standard dosing probably due to sorption, evaporation and limiting dissolution kinetics. Furthermore, passive dosing concentration-response curves were more reproducible and shifted towards lower concentrations. Results show that the response is clearly not only dependent on the potency of the compounds, but also on its supply, sorption and consumption during the assay. Passive dosing is a practical and ecologically more realistic approach than standard high throughput screening approaches for solid substances). First acute and chronic ecotoxicological tests with organisms of different trophic levels as proposed by OECD guidelines (algae, daphnids and fish-eggs) have been performed to verify the ‘poorly soluble approach’ according to the ETNCaP hypothesis.
regime explained about 65% of the observed variability in the data. Taking into account water temperature as an extra covariate increased the explained variability to almost 80%. The contribution of organic contaminants to the model fit was estimated to be 1%, but was not significant at a 5% level of significance. Most of the variability in the specific growth rate of the marine diatom was explained by the moment of sampling, the nutrient regime and the water temperature. More research is needed to confirm the preliminary results that are reported here.

TU135 Calibration and field evaluation of passive samplers as a new tool for monitoring pesticides in water
A Daneshvar, A. Lau, Swedish University of Agricultural Sciences; M. Günzli, SLU, Centre for Environmental Science; I. Krotz, Swedish University of Agricultural Science / Centre for Chemical Pesticides; L. Ahrens, Swedish University of Agricultural Sciences (SLU) / Dept of Aquatic Sciences and Assessment

The continuous emission of pesticides into the aquatic environment is posing a risk to wildlife and human health. Hence, there is a need for reliable monitoring tools for priority pollutants including pesticides in surface waters. Passive sampling techniques have been developed to improve water/quality assessment to determine time-integrated pesticide concentrations at ultra-trace levels and with minimum infrastructure. Five different types of samplers including i) polar organic chemical integrative/semiappraisal (POCIS)-A, ii) POCIS-B, iii) silicone rubber (SR), iv) Chemcatcher® SDB-RPS, and v)unchemcatcher® C18 were characterized in terms of sampling rates (Rs) and sampler-water partition coefficients (Kpw) for 124 pesticides. In addition, the performance of three types of passive samplers/ut (i.e. POCIS-A, SR, and Chemcatcher® C18) were investigated in two Swedish river systems over an period of six weeks, and passive sampler-derived concentrations were compared against time-integrated/negative sampling. The results showed that the passive samplers were capable to accumulate/pesticides with a wide range of Kpw. SR shows a better uptake for the more hydrophobic compounds (lower) whereas POCIS-A, POCIS-B, and Chemcatcher® SDB-RPS are more suitable for hydrophilic/compounds. The median Rs (L day-1) were 0.86 for SR, 0.22 for POCIS-B, 0.18 for POCIS-A, 0.05 for unchemcatcher® SDB-RPS, and 0.02 for Chemcatcher® C18. The median of log Kpw (L kg-1) were 4.78/for POCIS-B, 4.56 for POCIS-A, 3.17 for Chemcatcher® SDB-RPS, 3.14 for SR, and 2.71 for unchemcatcher® C18. Comparison of time-weighted average (TWA) concentrations for active/and/insensitive samplers in the field showed a good agreement. In total 52 pesticides were detected in the two rivers using active sampling, while 69, 58, and 32 pesticides were detected using the passive samplers/SR, POCIS-A, and Chemcatcher® C18, respectively. There were 4 pesticides (i.e., Dichloran selphos) that were detected only by the active sampler. On the other/hand, passive samplers detected in total 38 pesticides, which were not detected by the active sampler.InThe outcome of this study indicates that the passive samplers are suitable as a monitoring tool/inhowever, the reliability of the passive sampler-derived TWA concentrations need to be improved to apply passive samplers for regulatory purposes, for example in respect to the EU Environmental Quality Standards (EQS).
ion-exchange passive samplers and moss biomonitor (Hylocomium splendens)

B.G. Brumbaugh, US Geological Survey / Columbia Environmental Research Center
J. Arns, USGS Columbia Environmental Research Center; P. Neitlich, National Park Service

At remote locations, the use of conventional systems for monitoring deposition of atmospheric pollutants is often not practical; consequently, sampling methods that require minimal maintenance and no power for operation are needed in these areas. Mosses have been successfully used as biomonitor in many studies, particularly in northern Europe; however, few studies have examined relations between accumulation of atmospheric contaminants by mosses and actual deposition. We compared accumulation of selected inorganic atmospheric contaminants in mosses and three-year moss growth segments to contemporaneous deposition, the latter as measured by a combination of over-winter snowpack samples and specially adapted, passive ion-exchange collectors (IECs). Sampling was conducted at eight sites within interior Alaska, some of which were located near known sources of atmospheric contaminants so that depositional gradients might be measured. Snow and IEC extracts were analyzed for 10 elements (ammonium, cadmium, copper, lead, nickel, potassium, sodium, sulfur, zinc); major anions (chloride, nitrate, nitrite, sulfate); and a suite of trace metals including barium, chrom, copper, lead, nickel, and zinc. Annual growth segments of moss tissue samples were analyzed for total nitrogen, total sulfur, extractable cations and anions, and trace metals. Relations between moss concentrations and annual deposition as measured by IEC passive samplers and snowpack samples will be examined to develop exploratory models that characterize bioacumulation of atmospheric pollutants by the moss.

TU140 SPANISH AIR MONITORING OF ORGANOCHLORINE PESTICIDES, PCBs, PClD/Fs, dioxins and furans

P. Sanz, A. Torreg, J. Navarro, M. Martinez, CIEMAT

The Stockholm Convention (SC), is a global treaty to protect human health and the environment from Persistent Organic Pollutants (POPs). This Convention, coming into effect in 2004, requires Parties to take measures to eliminate or reduce the release of POPs into the environment. Since 2007, the Ministry of the Agriculture, Food and Environment manages the Spanish Implementation Plan (SIP). The main objective of the SIP is to establish a national monitoring network to characterize the current status and temporal trends of POPs and to evaluate the effectiveness of practices adopted to reduce POP emissions. In 2008 this network began monitoring POPs in air but, afterwards, it has been extended to other matrices such as soil and water. The aim of this study, framed in the SIP of the SC, was to investigate the POPs levels in Spanish regions. Concentrations of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides in air in the urban environment of Chittagong city (population approximately 4 million) and in vicinity of the ship-breaking areas. A sampling campaign was carried out using passive air samplers (PAS) based on polyurethane foam (PUF) at 23 sites in February 2013. PAS was selected as sampling material because their performance is well characterized as they have previously been used extensively for spatial mapping on both a regional and global scale. Performance reference compounds (PRCs) were furthermore used for back-calculating air concentrations. The results from the campaign in Chittagong will be compared and contrasted with concentrations of POPs in air reported from both developed and developing regions, and to discuss potential sources and source regions controlling the atmospheric burden of various POPs in the study area.

TU141 Occurrence of Persistent Organic Pollutants in Argentina, South America: use of passive atmospheric sampler (PAS) vs. pine needles

K.S. Mejilorganza, University of Mar Del Plata / Lab of Ecotoxicology and Environmental Health; R. Barra, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); M. Sila, Universidad Nacional de Mar Del Plata / Lab of Ecotoxicology; J. Roa, Instituto de Oceanografía, Universidad Nacional de Mar del Plata; M. Gomes Costa, Universidade Federal do Rio Grande / FURG / Laboratório de Microcontaminantes Orgânicos e Ecotoxicologia Aquática; M. Gonzalez, UNMdP / Lab Ecotoxicology and Environmental Pollution UNMdP/CONICET; F. Mitton, Universidad Nacional De Mar Del Plata; S. Giron, Universidad de Mar Del Plata CONICET; M. Silva Barni, Ciencias Marinas; F. Wania, University of Toronto at Scarborough / Department of Physical and Environmental Sciences; R. Barr, Universidad de concepcion / Facultad de Ciencias Ambientales Eula; G. Fillmann, Universidad Federal do Rio Grande / Instituto de Oceanograffy

Persistent organic pollutants (POPs), included in the Stockholm Convention, are characterized by global contamination, persistent in the environment and high toxicity. Atmospheric transport is responsible for pollutant dispersal over long distances. As part of the Latin American Atmospheric Passive Sampling Network (LAPAN), a monitoring program involving the use of pine needles and passive air samplers (PAS) was conducted. PAS using XAD-2 resin as a sorbent were deployed for three consecutive one-year periods (2010-2013) at seven sites in urban, rural, and remote areas throughout Argentina. The latitude of the sites ranged from Bahia Blanca (38°24′S; 62°16′W) in Buenos AiresProvinceto Río Gallegos (51°38′S; 69°14′W), the southernmost site of Argentina. The main objectives of the project were: 1- To gather new data on atmospheric pollution (at improved spatial and temporal resolution); 2- To assess the influence of local and global sources by deploying PAS in rural, industrial, urban and remote areas; 3- To compare the occurrence and distribution of POPs in PAS and pine needles. Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polynobrominated diphenyl ethers (PBDEs) were determined by GC-ECD and GC-MS and concentrations expressed in units of pg/m³. At sites at lower latitudes, which are close to agricultural zones endosulfans (150 pg/m³) were
The predominant POPs in air, with a relative abundance of a-f-isomers similar to that of the technical mixture (7:3). DDT levels were also higher at lower latitudes with a predominance of the parent DDT (100 pg/m³). On the other hand, PCBs concentrations, ranging from 3.7 to 46 pg/m³ increased with latitude with higher average values at Rio Gallegos with a predominance of lower chlorinated congeners (#18, #44, 52, 118). The industrial area of Bahia Blanca appears to be a source of PBDEs (3-3 pg/m³), increasing in strength since from 2010 to 2013. Our previous results for PBDEs in air showed a similar partition distribution pattern as the PAS. However, metabolites (DDE, endosulfan sulfate, heptachlor epoxide) were more dominant in pine needles. Although both samplers were useful to assess the atmospheric pollution by POPs, an integrated study using PAS and biological samplers is suggested in order to understand the behavior of these compounds in the atmosphere.

TU146 Passive sampling of ionized pharmaceuticals using C18-based SPME coatings H. Pettenkofer, Institute for Risk Assessment Sciences; S. droge, Utrecht University / TRAS; S. Bohm, Austrian Border Region Institute; J.L. Hermons, Utrecht University / Institute for Risk Assessment Sciences

Commonly used extraction phases in solid-phase microextraction include neutral coatings such as polyacrylate (PA) or polydimethylsiloxane (PDMS). However, these coatings only show high sorption coefficients for neutral analytes, with ionizable compounds only sorbing after matrix-modifying steps to ensure a large neutralization. Previously, studies have shown that the C18 fiber (poly(dimethylsiloxane)-coated C18), and strong cation exchange (SCX) groups showed limited pH-dependent sorption for ionizable compounds, indicating that these coatings could be beneficial for charged species especially. We compared sorption of pharmaceuticals to two C18-based SPME coatings. Sorption of four model compounds was studied for both the analogous C18 fiber and the C18/SCX fiber. The chosen model compounds are all alkaloids: amitriptyline (log Kow, 3.48, pKa 9.7), amphetamine (log Kow, 1.80, pKa 9.9), diazepam (log Kow, 3.08, pKa 2.9) and tramadol (log Kow, 2.45, pKa 9.2). Our results indicate that sorption to both C18 and C18/SCX coatings is only slightly pH-dependent. Furthermore, with sorption coefficients for cationic compounds still high at very low pH, this indicates that it is not the remaining neutral fraction that has high affinity for C18-based coatings but that also the charged species has similar affinity for these fibers. The C18/SCX coating showed somewhat higher sorption affinities for cationic compounds compared to C18 coating alone. However, sorption of charged species to the C18/SCX fiber decreases with increasing ionic strength, whereas the C18 coating might be less affected by the competitive effects of salts. To prove that sorption of cationic compounds to the C18 coating was not driven by available silanol groups, anionic diflucan (log Kow, 2.16, pKa 3.8) was used as a model compound. Diflucan is an anti-fungal drug only slightly pH-dependent. This proves that completely ionized compounds (albeit cationic or anionic) are able to sorb to C18-based coatings. Furthermore, diflucan sorption to the C18/SCX coating is comparable to sorption to the C18 fiber at pH 7.4, showing the large contribution of C18 in the C18/SCX fiber to the sorption of diflucan. It is clear from our data that C18-based SPME coatings show improved sensitivity over conventional SPME coatings, can be applied without matrix-modifying steps, and are able to sorb both neutral and charged drug species. These insights highly contribute to the use of SPME in the measurement of sorption processes.

TU147 Developments, Applications and Perspectives of the Passive Water Sampler for organics - o-DGT C. Chen, A.J. Sweetman, H. Zhang, Lancaster University / Lancaster Environment Centre; M. Bowkett, TeTelLab; K.C. Jones, Lancaster University / Lancaster Environment Centre

Passive water sampling has several advantages over active methods: it provides time weighted average (TWA) data, saves time and money and can yield highly resolved data. A novel passive water sampler (called o-DGT) for organic chemicals (antibiotics as model compounds) based on diffusive gradients in thin-films (DGT) has been developed and tested. This sampler has been used to for a wide range of applications including chemical fate in wastewater treatment plants (WWTPs) and for ionizable partitioning compounds in freshwater and estuaries. This passive sampling technique has been further explored and applied to soils for in situ measurement of the concentrations and fluxes of antibiotics in soils and for understanding the desorption kinetics in soils. DGT is also being used for similar studies in sediments. The development of this technique for other groups of chemicals is ongoing, with other pharmaceuticals, personal care product ingredients (PPCPs) (including some parabens, estrogen, bisphenol A, and triclosan) and pesticides being assessed. Recent results show that PPCPs can be measured with this sampler under laboratory test conditions, but also in the field (i.e. WWTPs) by modifying the binding agent, diffusive gels and filter membranes (unpublished data). Further development and validation of the passive sampler which can be used to determine a wide range of priority substances under the Water Framework Directive (WFD) is an important development goal for our research. Overall, o-DGT is a promising tool for understanding the fate and behaviour in situ approach for assessing the availability of polar organic chemicals in environmental water. Further development and calibration, o-DGT might be also be applicable to persistent organic pollutants (POP) to assess their fate, behaviour and bioavailability.

Acknowledgement – We thank EPSRC and TeTelLab for their financial support.


TU148 NAKED PASSIVE SAMPLING OF POLAR ORGANIC CONTAMINANTS

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USING THIN SILICONE SHEETS IN A WASTEWATER TREATMENT PLANT AND RECEIVING RIVER
K.E. Smith, Korean Institute of Science and Technology Europe / Convergence Environment Team; Y. Jeong, KIST Europe

The advantages of passive sampling include the provision of equilibrium or time-weighted averages of the dissolved concentrations, the possibility of in situ sampling and no power requirements. In addition, the selective up-concentration of the contaminants compared to many dissolution methods results in relatively clean samples with high levels which simplifies analysis. Silicone is commonly used for hydrophobic organic contaminants due to their high partitioning into this polymer. However, it has a lower relative affinity for more polar contaminants (including many pharmaceuticals, biocides etc.) which can lead to problems with analytical detection. To some extent this limitation can be solved by using larger silicone volumes in conjunction with highly sensitive analytical methods. To ensure high exchange kinetics, in this study “naked” passive samplers were directly exposed to waste and river waters. These consisted of thin 100 cm² PDMS sheets with thicknesses of 96 and 203 µm enclosed in a fine copper mesh to limit biofouling and provide rigidity. Prior to deployment, the PDMS sheets were spiked with labelled surrogates of the target contaminants as performance reference compounds. The passive samplers were deployed for week long periods over 1 month in the inlet and outlet of a wastewater treatment plant, as well as in the Saar River which receives the treated effluent. After recovery the silicone sampling sheets were solvent extracted, blown-down and analysed by LC-MS/MS for a set of compounds based around the Water Framework Priority list. In addition to hydrophobic contaminants such as PAHs, a range of polar contaminants were routinely detected including pharmaceuticals (e.g., atenolol, metoprolol and carbamazepine) and biocides (e.g., isoproturon, diuron, cybutryne). The similar polymer concentrations measured for both sheet thicknesses, along with the almost complete disappearance of the labelled surrogates, indicate that equilibrium was almost reached. This allows the measured polymer concentrations to be expressed in comparison into the dissolved concentrations using partitioning ratios. Therefore, this study shows that silicone can be used for the passive sampling of polar organic contaminants in waste and surface waters.

TU149 Partitioning kinetics of polar organic contaminants to OASIS HLB as a function of pH and ionic strength for optimization of passive sampling and solid phase extraction approach
Y. Jeong, KIST Europe; K.E. Smith, Korean Institute of Science and Technology Europe / Convergence Environment Team; A. Schaeffer, RWTH Aachen University / Institute for Environmental Research

The performance of OASIS HLB for the collection of polar contaminants assumes an ever increasing importance, and here sampling is the initial step. Passive sampling is regarded as a promising technique for the monitoring of water quality providing time-weighted averages of the dissolved concentrations which are considered to be the bioavailable concentrations driving uptake and toxicity, the possibility of representing the sampling phase results in in situ sampling, and no power requirements. Furthermore, the selective up-concentration of the contaminants compared to many dissolution methods results in relatively clean samples with high levels to simplify analysis. Increased concerns about polar organic chemicals in aquatic environments led to the development of the polar organic chemical integrative sampler (POCIS). This consists of an adsorbent polymer enclosed between two membranes. POCIS are typically deployed in kinetic sampling mode, where uptake is linear and proportional to the dissolved concentration of contaminants. The rate of both prior to dissolution phase is determined by factors such as the properties of the target chemicals as well as POCIS materials and the hydrodynamic conditions. Therefore, one of the key factors determining the accumulation behavior of polar organic contaminants in the POCIS relates to their sorption behavior to the adsorbent polymer. OASIS hydrophilic lipophilic balance (HLB) is the one of the most widely used sorbents in the POCIS, yet a detailed understanding of the partitioning behavior of different polar organic contaminants to this porous polymer is missing. Therefore, a miniaturised batch set-up was implemented in HPLC viaals allowing the sorption of a wide range of polar organic contaminants at environmentally relevant levels to OASIS HLB to be measured. Initially equilibrations experiments were performed, and based on these the equilibrium sorption of polar organic chemicals as a function of concentration, temperature, pH and ionic strength determined. Increasing concentrations led to a flattening of the sorption isotherms, likely due to limited availability of sorption sites. As expected, increasing temperature led to a decrease in sorption, with the magnitude of this depending on the compound. The effects of pH and ionic strength were complex, leading to an increase, decrease or having no effect on the sorption. The partitioning ratios of polar organic chemicals to OASIS HLB are discussed in terms of the properties of the chemical, sorbent and aqueous solvent.

TU150 Passive sampling of cationic surfactants in sorption studies with various environmental matrices
N. Timmer, J.L. Hermens, Utrecht University / Institute for Risk Assessment Sciences; s.t. droge, Utrecht University / IRAS

Cationic surfactants are a challenging but poorly studied group of environmental contaminants that are worldwide observed to accumulate in sediments and soils that receive sewage waste material, either via waste water effluents or via sludge disposal. Cationic surfactants can sorb to hydrophobic phases as well as to matrices containing negatively charged surfaces – such as humic acids, fulvic acids and clay particles. A major analytical challenge is that long chain cationic surfactants strongly sorb to most lab surfaces – i.e. glass vials, pipet tips and microtiter plates – rendering the measurement of actual freely dissolved concentrations problematic. For this study, a group of alkylated primary, secondary, tertiary and quaternary ammonium compounds were selected for the development of a fast and efficient sampling method. Selected compounds structures. WCX-SPE and 7 μm coated polyacrylate coated SPEME-fibers were used to calculate fiber partitioning coefficients. These SPEME-fibers were subsequently used to determine free concentrations of cationic surfactants in different media and under different conditions. In addition, SPEME-fibers were used to determine sorption affinity for a small set of surfactants to different types of sediment (i.e. clay, sandy, organic rich), LUFAs soils, and for various soil/sediment constituents (Aldrich humic acid, kaolinite, illite), revealing the importance of various individual soil components in controlling bioavailability in contaminated ecosystems.

TU151 Sampling polyhexamethylene guanidine (PHMG) aerosols using Eosin Y coated glass beads
S. Choi, S. Park, H. Kang, Korea University; K. Lee, Korea Institute of Toxicology; J. Kwon, Korea University / Division of Environmental Science and Ecological Engineering

Aerosols caused by the use of polyhexamethylene guanidine (PHMG), a general-purpose chemical germicide used as a humidifier disinfectant in Korea, have raised concerns about bioaerosols in indoor environments. A sampler capable of accumulating PHMG from aquatic aerosols was developed as an alternative to low-volume air samplers. This sampler was prepared by placing glass beads coated with Eosin Y (2-(2,4,5,7-tetramethyl-3-oxo-3H-xanthen-9-y)-benzoate) in a custom-made plastic holder. Passive sampling rates, measured in a bench-top exposure chamber at two different aqueous PHMG aerosol generation rates, were found to be independent of the experimental conditions. This suggests that the capacity of the sampler to accumulate the PHMG aerosol was sufficient for the sampling duration tested. However, the passive sampling rate was 0.0065 L/h, which is lower than the typical human breathing rate and inadequate for quantitative instrumental analyses at low concentrations in indoor air. A 25-fold enhancement of the sampling rate was achieved by forced convection using a commercial battery-operated fan above 2000 rpm. This suggests that it would be possible to utilize this sampler for quantifying time-integrated concentrations of PHMG aerosols in the air.

TU152 Silicone passive samplers for organic pollutants in water: Utilizing accelerated solvent extraction (ASE) for purification and extraction
B. Brockmeyer, German Federal Maritime and Hydrographic Agency (BSH); N. Theobald, BSH / Marine Chemistry

In recent years, silicone passive samplers have gained increasing attention as single phase, practical and robust samplers for monitoring of organic contaminants in the aquatic environment. However, analytical challenges arise during extraction of analytes as silicone associated oligomers are co-extracted and interfere with the chemical analysis. Thus, solvent and time-consuming precleanup methods are necessary, both prior to extraction and before instrumental analyses at low concentrations in indoor air. A 25-fold enhancement of the sampling rate was achieved by forced convection using a commercial battery-operated fan above 2000 rpm. This suggests that it would be possible to utilize this sampler for quantifying time-integrated concentrations of PHMG aerosols in the air.

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TU153 Effect of environmental temperature on the loss of PAHs in passive samplers
N. Dominguez, URVIISSP; M. Schuhmacher, Rovira i Virgili University / Laboratory of Toxicology and Environmental Health; M. Nadał, University Rovira i Virgili / Laboratory of Toxicology and Environmental Health; Y. Jeong, KIST Europe; K.E. Smith, Korean Institute of Science and Technology Europe / Convergence Environment Team; M. Nadal, University Rovira i Virgili / Laboratory of Toxicology and Environmental Health; M. Nadał, University Rovira i Virgili / Laboratory of Toxicology and Environmental Health

Fatalities caused by the use of polyhexamethylene guanidine (PHMG), a general-purpose chemical germicide used as a humidifier disinfectant in Korea, have raised concerns about bioaerosols in indoor environments. A sampler capable of accumulating PHMG from aquatic aerosols was developed as an alternative to low-volume air samplers. This sampler was prepared by placing glass beads coated with Eosin Y (2-(2,4,5,7-tetramethyl-3-oxo-3H-xanthen-9-y)-benzoate) in a custom-made plastic holder. Passive sampling rates, measured in a bench-top exposure chamber at two different aqueous PHMG aerosol generation rates, were found to be independent of the experimental conditions. This suggests that the capacity of the sampler to accumulate the PHMG aerosol was sufficient for the sampling duration tested. However, the passive sampling rate was 0.0065 L/h, which is lower than the typical human breathing rate and inadequate for quantitative instrumental analyses at low concentrations in indoor air. A 25-fold enhancement of the sampling rate was achieved by forced convection using a commercial battery-operated fan above 2000 rpm. This suggests that it would be possible to utilize this sampler for quantifying time-integrated concentrations of PHMG aerosols in the air.
variables such as wind speed and temperature, which may significantly affect the uptake capacity of these devices, has not been fully investigated. Previous studies have analysed the role of temperature on the retention capacity of semi-volatile organic compounds (SVOCs) by PUF-PAS, with polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides and polychlorinated biphenyls having attracted most of the attention. The results of these studies show the increase of the temperature in a climate chamber reduces their sorption rates. Therefore, metabolism, bioavailability, and the effects of environmental conditions on the sorption behavior. Furthermore, they are mainly in the form of fine beads, making their handling complicated. Therefore, to combine the desirable properties of both polymer types, this study followed a simple approach of casting OASIS HLB® beads with PDMS silicone at different ratios. Subsequently, the partitioning behavior of a wide range of polar organic contaminants (biocides, pharmaceuticals, hormones etc.) was determined at 4°C, 20°C and 37°C. Compared to PDMS alone, the mixed polymer showed a much increased partitioning between the polar organic compounds due to the increased affinity of the embedded OASIS HLB®. Furthermore, in most cases desorption was reversible, and immobilizing the 5 μm OASIS HLB® beads in the PDMS greatly simplified the handling. Therefore, the simple approach of mixing polymers with different chemistries offers a practical approach to tune the sorption affinity for a wider range of organic contaminants for passive sampling and dosing applications.

TU156 Development of a passive sampler for the monitoring of nanosilver in environmental waters and wastewaters
L. Shen, Li Shen / Department of Chemistry; C.D. Metcalfe, Trent University / Ontario Quality Centre; V.V. Yargoeu, McGill University / Chemical Engineering

As a result of expanding use, nanomaterials such as nanosilver (AgNP), are being continuously released into the environment through production, usage, and disposal. Research is currently needed for the development of cost-effective methods to quantify AgNP in various environmental compartments in order to fully evaluate the exposure and risks on the environment and public health associated with this emerging nanoscale particle. Currently, various methods are used for the determination of AgNP, but few have focused on the development, calibration and application of passive sampling to the detection of AgNP and the measurement of low concentrations in complex matrices. Our recent work has allowed us to calibrate a sampler containing carbon nanotubes (CNT) in powder form as the sorbent for AgNPs in aqueous matrices. The calibrated CNT samplers have been applied to determining the concentrations of AgNP in a water and pesticide treatment plant (WWTP) and in a river upstream and downstream of the WWTP discharge point. Results showed a concentration of AgNP of 0.3 ppb in the treated effluent with a limited impact on the concentration in the receiving stream; 1.0 ppb upstream and 1.3 ppb downstream of the wastewater discharge point. Data will also be presented for samplers deployed in Lake Ontario near WWTP discharges and in a lake experimentally dosed with AgNPs. This study demonstrated the potential of the CNT samplers as a tool for monitoring the concentration of nanomaterials in wastewater and in surface waters impacted by wastewater discharges.

TU157 Comparison of the Uptake of Perfluorinated Alkyl Substances by Carrot Plants and Passive Samplers
E. Bizkarguengua, I. Zabaleta, Analytical Chemistry; A. Vallejo, University of the Basque country UPV/EHU; A. Prieto, University of the Basque country UPV/EHU / Analytical Chemistry; L. Fernández, O. Zuloaga, University of the Basque country UPV/EHU

The aim of this work was to study the uptake of perfluoroalkyl substances (PFASs) such as perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonamide (FOSA) by two carrot varieties (Chantenay and Nantes). Carrots were grown in two types of compost-amended soils (characteristics of soil are the following, pH: 7.2 and 5.68 and TOC (%): 2.26 and 53.32 for soil 24 and substrate, respectively) fortifed at 500 ng g⁻¹ concentration level (two pots for fortified samples and one pot for unfortified samples for each analysis). PFOS was found in the carrot leaves compared to the peel and core for 3 different passive samplers (POM, PES and SR) with the three target analytes. Besides, degradation of PFOSA to PFOA was observed in all the experiments carried out (two different types of soils and two different varieties of carrot). For PFOS a higher BCF was observed for the soil with the lowest content in organic matter, while the differences were not so significant for PFOA. The three different passive samplers (POM, PES and SR) were also tested to prove the availability and compatibility of the passive samplers observed in the passive samplers with the BCFs obtained in the carrots. PFASs were detected in all the passive samplers except SR. Therefore, comparison between BCFs obtained for PES and POM and BCFs of all the sum of the three compartments of the plant (sum of BCFₑₑₑ, BCFₑₑₑₑ and BCFₑₑₑₑₑ) were carried out. No correlation was observed between BCFₑₑₑₑₑ and BCFₑₑₑₑₑ, while a linear trend was achieved for BCFₑₑₑₑₑ and BCFₑₑₑₑₑ from the degradation of FOAS (r² = 0.81) in the case of PES passive sampler. Besides the slopes of the correlations between the uptake of PES and the BCF for POFOS coming from the amended soils fortified with PFOS (0.66) and fortified with FOAS (1.08) were statistically comparable. To our knowledge, this is the first time that the uptake of PFASs by carrots is satisfactorily correlated with the uptake in a passive sampler.

TU158 Microbial Passive Samplers: How reliable?
J. Monier, C. Malandain, C. Baguelin, O. Sibourg, ENOVEO

Successful bioremediation of subsurface environments, such as contaminated soil
or groundwater, can depend on a good understanding of microbial degradation
processes. Taking into account the complexity of interactions that occur between
the solid matrix, indigenous microorganisms and pollutants, initial on-site
characterization and in situ monitoring of microbial communities over time are
essential. One of the major challenges with subsurface systems has been the
development of sampling techniques for microbiological investigations. Reliable
sampling is highly critical since detection of microbes and/or their expression, and
the quantification of genes involved in degradation processes are often used to
design and monitor remediation processes. Conventional (active) sampling usually
relies on the collection of individual spot samples and may often lead to an
underestimation of the abundance and diversity of the community as well as an
important variability. The objective of our work was to develop and optimize tools
for reliable on-site passive sampling of microbial communities in non-destructive
manner. Different matrices ranging from activated carbon to coarse sand were
tested for enrichment of bacterial growth in monitoring wells. Samplers were
maintained over 30 days and the microbial communities enriched on the different
matrices compared to the communities of the surrounding soil and interstitial water
using molecular tools (e.g., Next Generation Sequencing, RISA, Phylogenetic
microarrays). Results obtained showed that the amount of biomass and structure of
the communities are different on the different matrices tested. Some solid supports
seem less adapted than others in order to sample in a reliable manner the microbial
communities present in the surrounding water and soil. Therefore, the choice of the
matrix selected to passively sample subsurface microbial communities is highly
critical and some material are to be avoided. Data obtained with the different
matrices in different environments will be presented and the advantages and
limitations of such approach for different applications will be discussed.
TU159
Lichen bioaccumulation data at support of PM10 diffusional models: the case
study of a coal power plant in a highly heterogeneous territory
L. Fortuna, Department of Life Sciences; G. Adami, University of Trieste /
Department of Chemical and Pharmaceutical Science; M. Tretiach, University of
Trieste / Department of Life Sciences
Diffusional models are requested by standard authorization processes for new
industrial plants or modification of old ones, but their limit is that they are validated
only rarely through the cross-check with further environmental data (e.g. direct
field measurements of pollutant concentration). In this work we show that lichen
bioaccumulation data can be used for the validation of diffusional models of PM 10
concentrations at ground level. The study was carried out in a low-elevation
territory (Monfalcone, NEItaly), characterized by high land use heterogeneity and
high anthropic pressure. The tested models describe the PM 10 fall-out of a
medium-size coal power plant (models a, b, respectively), and that of all the
industrial plants present in the survey area (model c). They were specifically
developed for obtaining the original licence (in 1996 and later in 2009; models a,c )
or as a consequence of a recent environmental litigation, opposed by a citizen
committee (2014, model b). The lichen survey, was based on a simple systematic
sampling, in order to strongly reduce the subjectivity of the samplers: the study area
(176 km2) was divided into 44 Primary Sampling Units (PSU) of 4 km2 each. The
lichen sampling was limited to 10 PSUs, of which five, indicated as “potentially
polluted” (PP), corresponded to the maximum fall-out described by models a (3
PSUs) or c (2 PSUs), and other five, indicated as “potentially unpolluted” (PU, i.e.
the controls), were selected externally to the plumes of the two models, according to
the same land use of their PP counterparts. In each PSU one to three samples of
Flavoparmelia caperata and Xanthoria parietina, co-occurring on one and the
same tree were collected, for a total of 42 samples, that were prepared and digested
according to standard protocols. Element concentrations were measured by
ICP-AES, GFAAS, and DMA. The comparison between the bioaccumulation data
of PPs and PUs revealed that two PPs of model a did not differ statistically from
their putative controls, while the third one showed a lower content for 13 elements
with respect to its control. This contradiction was substantiated through the
comparison with the outputs of model b. In fact, that specific “control” PSU,
purportedly unpolluted according to model a, was found to correspond to a
significant portion of the fall-out plume described by the more recent model,
elaborated according to more sophisticated algorithms.

Mercury Pollution: Chemistry, Remediation and Policy (P)
TU160
TOTAL GASEOUS MERCURY CONCENTRATION AND LICHENS
BIOACCUMULATION IN THE NORTHERN ADRIATIC COASTAL
AREA (GULF OF TRIESTE, ITALY)
A. Acquavita; R. Grahonja, M. Pasquon, F. Tamberlich, G. Mattassi, ARPA FVG
The Gulf of Trieste (NE Italy) and the adjacent Marano and Grado Lagoon have
been affected by significant mercury (Hg) contamination mainly for the existence
of mining activities in Idrija (Slovenia) and, to a lesser extent, the chlor-alkali plant
in Torviscosa. Due to its volatile nature, Hg emission from terrestrial and aquatic
surfaces is an important part of its biogeochemical cycle. Contaminated sites and
surrounding enriched soils can be regarded as substantial atmospheric mercury
emission sources. Lichen species have proven to be a good indicator of long-term
Hg dispersion patterns around an industrial facility and in other Hg dispersion
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studies. In this work, a preliminary assessment of Hg bioaccumulation and its
relationship with gaseous mercury levels was conducted. Mercury determination in
lichens was performed by means of a mercury direct analyzer (DMA-80,
Milestone), whereas measurements of Hg0 were made using an RA-915+ Lumex
Zeeman portable mercury spectrometer. Concentrations of Hg in in situ lichens
ranged from 50 to 150 ng g-1, which are typical values of uncontaminated sites.
However, some hotspots were recorded in near the chlor-alkali plant and Grado
seaside (~ 400 ng g-1). Mercury in air showed an average concentration of about 3.0
ng m-3, similar to that reported in uncontaminated area, rarely exceeding 10 ng m-3.
It is well known that Hg bioaccumulation in lichens from the atmosphere depends
on the ambient levels, age of the lichens and the physiological conditions. In this
work no significant correlation was found , thus suggesting that a more exhaustive
survey of biological and soil matrices is necessary in order to increase behaviour of
Hg in these contaminated sites.
TU161
Mercury and POPs in marine debris from a remote coral reef island in the
Indian Ocean
H. Bouwman, North-West University / Environmental Sciences and Developmen;
M. Krátká, Masaryk University / RECETOX Research Centre for Toxic
Compounds in the Environment; R. Choong Kwet Iyve, University of Mauritius /
Chemistry; H. Kylin, Linköping University / Department of Thematic Studies
Environmental Change; J. Klanova, Masaryk University / RECETOX Research
Centre for Toxic Compounds in the Environment
The real and potential impacts of marine debris is attracting much attention due to
the amounts involved and the large areas affected. This presents the potential of
facilitated long-range transport of accumulated persistent organic pollutants
(POPs), the inherent organic chemicals, and heavy metals such as mercury. We
found large amounts of plastic debris (± 3.1 million pieces) on the beaches of the
remote atoll of St Brandon’s Rock (SBR) in the Indian Ocean.The brand names on
the debris indicate origin from countries bordering the East Indies and northern
Indian Ocean. We predicted that islands can scrub debris from ocean currents and
that UV, wave action and biological action will release the pollutants to
contaminate coral reefs. We collected small pieces of plastics, coral rubble, coraline
beach sand, and sand from tern breeding colonies, which were analysed for mercury
and POPs. Concentrations and compositions of the OCPs, new BFRs, and PFCs
were quite variable within and between the different matrixes. Plastic debris pieces
generally had the highest concentrations of all compound classes. However, for all
classes, there were natural matrix samples that had higher concentrations. HCHs
dominated the OCPs. Most were α-HCH. Of the DDTs, p,p’-DDT seemed to
dominate in the plastics, with almost no p,p’-DDE to speak of. No DDTs were
found in the coralline matrixes. Plastics had very variable compositions and
concentrations of BFRs, with decabromodiphenylethane (DBDPE) and
1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) dominating, but the coralline
matrixes almost exclusively had syn-dechlorane plus and anti-dechlorane plus.
Perfluorobutericacid (PFBA) and perfluoropentanoicacid (PFPA) dominated the
PFAS in all matrixes, and generally had the highest concentrations in plastics.
PFOS and PFOA occurred in relatively small amounts compared to the other PFAS
in all samples. The concentrations of mercury was highest in those plastics that had
them, but levels were quite variable within each matrix. The heterogeneity of
compositions and concentrations of pollutants in the coralline samples
indicate plastics as sources rather than derived from a homogenous background. It
is clear that plastics can accumulate pollutants such as mercury from seawater and
transport them to remote “pristine” regions. Small island states may whish to take
note when considering the Minamata Convention.
TU162
A mechanism for the reduction of HgCl2 on environmental surfaces
G. Brown, John Jay College of Criminal Justice / Sciences; A. Ho, University of
California Davis / Plant Biology; R. Khusial, John Jay College of Criminal JusticeCUNY / Sciences; A. Carpi, John Jay College
The mechanism of the reduction of divalent mercury (Hg) in the environment and
its subsequent emission from soil into the atmosphere is not well understood.
Reduction and emission is known to be influenced by many environmental factors
such as temperature, light, or microbial activity; however, the nature of these effects
are not known. Identifying the mechanism by which Hg species are reduced in soil
can shed light on the dynamic biogeochemical cycling of Hg and possible
remediation of contaminated sources. In this study, the mechanism of the reduction
of inorganic mercury (Hg2+) to elemental mercury (Hg0) on environmental surfaces
was investigated through laboratory analysis and computational modeling.
Laboratory analysis was carried out under a laminar flow hood using a Tekran 2537
Mercury Vapor Analyzer (Tekran Inc., Toronto Canada) which detects trace levels
of Hg0 on a scale of ng/m-3. Elemental mercury emissions were measured from
HgCl2 treated samples of inert materials even in the absence of third party
intermediaries. Hg0 emissions were seen to be primarily driven by Ultraviolet-B
(UVB) radiation. Emissions were seen to be independent of temperature in the dark,
but correlated with ambient temperature in the presence of light. Gaussian
computational modeling shows that the molecule exhibits a transition state when
the chlorines are excited such that they bend to 62 degrees. This transition state
occurs at an energy of 99.1 kcal/mol, which corresponds to light in the UVB range
(288 nm) of the electromagnetic spectrum. Thus, this work will present a

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preliminary mechanism for the reduction of HgCl₂ in the environment and subsequent emission of Hg⁰ from HgCl₂-treated soils.

TU163 Effects of wildfires on mercury mobility in eucalypt and pine forests I. Campoo, Universidade de Aveiro / Department of Environment; C. Vale, IPMA Instituto Portugueses do Mar e da Atmosfera; N. Abrantes, University of Aveiro / CESAMDAO; J. Keizer, Department of Environment Centre for Environmental and Marine Studies CESAM University of Aveiro Aveiro Portugal; P. Pereira, Department of Biology and CESAM Wildfires and subsequent rainfall have a major impact on forest ecosystems and on the redistribution of trace elements, which are mobilized from soil and ashes. In particular, forest fires can alter the mobility of mercury (Hg) through its release due to its volatilisation and toxicity. The present study addresses this concerning issue by comparing the concentrations of Hg in topsoil (0-2 cm) and ashes in burnt and long-unburned forest plantations (Ermita and São Pedro do Sul, North-centre of Portugal). Fire was classified as moderate in Ermita and moderate to high severe in São Pedro do Sul. Sampling collection took place immediately and 4 months after the fire, the latter following an episode of heavy rainfall. Mercury analysis was performed in an Hg analyser (AMA 254 LECO Instrument), in which samples were thermally decomposed by controlled heating. The final decomposition products were passed through an Hg amalgamator heated to 700 °C and Hg²⁺ was released and detected by absorption spectrometry at 254 nm. This technique allows eliminating the digestion procedure prior to analysis and is free from matrix interferences. The principal results obtained in this study are: (1) 30% of the Hg retained in eucalypt soils was released by the fire, corresponding to a loss of 1.0 - 1.1 g Hg per hectare of burnt soil; (2) levels of Hg in burnt eucalypt soils doubled the values registered in burnt pine soils for both areas; the Hg concentrations in ash revealed a similar pattern; (3) Hg concentrations in both soils and ashes differed significantly between the two burn areas, possibly due to differences in fire severity or combustion of 1.0 - 1.5 g per kg ash; (4) high enhancement of 0.5 g Hg per hectare in the soil washed out. The difference between the previous estimations, 0.5 g per hectare, corresponds to the quantity of Hg transported to the surrounding areas or eventually introduced into aquatic systems. Our results highlight that wildfires and subsequent rainfall play a key role in the mobilisation of mercury in the environment and point out the importance of further studies to assess the impact of Hg in aquatic systems downstream to burnt areas. Keywords: Wildfire; Runoff; Mercury pathway; Fire severity

TU164 Effect of different molecular weight fraction of dissolved organic matters on photo-degradation of MeHg in water M. Kim, Seoul National University / Dept of Environmental Health; A. Won, Department of Environmental Health; T. Kim, Seoul National University; K. Zoh, Seoul National University / Department of Environmental Health Methyl mercury (MeHg) is a potent neurotoxin that may pose a threat to ecosystem viability and human health. In aquatic systems, the photolytic degradation of MeHg is considered as a bioremediation process. However, the photolytic reactivity of the MeHg and its degradation rate is also affected by photo exposure leading to changes in DOM composition (e.g. loss of aromaticity), which can affect its bioavailability and its role in overall mercury (Hg) cycling. Recent studies have shown that DOM plays an important role during photo-degradation of MeHg. Some studies reported that MeHg degradation rates decreased with increasing DOM concentration due to reduced penetration of light, while other studies reported that the presence of DOM enhanced the rate of MeHg degradation. Within this study, we investigated the effect of different molecular weight fractions of DOM on the photo-degradation of MeHg by (1) separating DOM into three fractions by molecular weight: MW < 3500 Da, 3500 Da < MW < 10000 Da, and MW > 10000 Da, (2) examining the effect of different DOM fractions on the photo-degradation under various conditions, and (3) determining the photo-degradation kinetics of MeHg by different DOM fractions. The results showed that the humic acid portion with 3.5 K < MW < 10 K was most enriched, accounting for 62.8% of total DOC content. Among the fractions of humic acid, the smallest molecular weight fractions (MW

TU165 MERCURY IN MUSHROOMS: CONTENTS AND BIOACCESSIBILITY D. Villalba, Consejo Superior de Investigaciones Científicas / Conservación y Calidad de Alimentos; C. Jadaí, C. Crespo, Consejo Superior de Investigaciones Científicas; S. Serrano; V. Devesa, Consejo Superior de Investigaciones Científicas Carlos Jadaí, Cristina Crespo, Susana Serrano, Vicenta Devesa, Dinoraz Vélez* Instituto de Agroquímica y Tecnología de Alimentos (IAATA-CSIC). Av. Agustín Escalderón nº 7, 46980 Paterna, Valencia (Spain). Corresponding author (*): phone +34 963 900 022, fax +34 963 636 301, email deniz@iaata.csic.es Food is the main source of exposure to mercury for most of the population. The most problematic food matrices are seafood products, in particular large predatory fish (swordfish, tuna and albacore), which present contents that may exceed the maximum allowed in the regulations. It has also been detected high contents of mercury in products of plant origin, especially mushrooms and derivatives. These foods are not controlled by the health authorities, as there is no legislation; however, in some cases they may suppose a risk for regular consumers. In general, food safety assessment of mercury in foods has been carried out on the raw product. However, the evaluation of the risk from mercury concentrations in raw food might be modified if cooking and bioaccessibility (the contaminant fraction that solubilises from its matrix during gastrointestinal digestion) and becomes available for intestinal absorption is taken into account. The aim of this study was to assess the contents of mercury in fresh, canned and dehydrated mushrooms marketed in Spain and determine the effects of cooking and of the process of gastrointestinal digestion on the mercury associated with their intake. Mercury contents in dried mushrooms are larger (< LQ = 4.4 mg/kg) than in fresh (< 0.04 mg/kg) and predominate in organic matrixes that are the most abundant species. Cooking entails reductions in the contents of mercury, which is solubilised into the liquid of hydration or cooking. Despite these reductions, the consumption of a dietary portion (30 g) of dehydrated mushrooms after cooking can exceed the daily intake for inorganic mercury recommended by the World Health Organization (0.43 µg/g body weight). The gastrointestinal digestion process produces a decrease in solubilisation of most of the mercury present in the cooked mushrooms (bioaccessibility < 25%), which diminish the risk associated to mushroom consumption.

TU166 Is Hg uniformly distributed among soil aggregate size fractions? A case study on podzol horizons in sub-Antarctic forest soils of Tierra del Fuego (Argentina) A. Gómez-Armero, University of Vigo / Plant Biology and Soil Science; M. Pérez-Mills, University of Vigo; X. Pontevedra-Pombal, University of Santiago de Compostela; P. Pérez-Rodríguez, M. Arias-Estévez, University of Vigo; S. Dioldato, CADIC-CONICET; A. Moretto, CADIC-CONICET-Universidad Nacional de Tierra del Fuego, E. García-Rodeja Gayoso, Universidad de Santiago de Compostela; L. Cutillas Estévez, University of Vigo. Although the atmosphere plays a key role in the global Hg cycle, this metal has a limited time of residence before its deposition in the soil surface. Soils are recognized as the largest Hg sink, accounting up to 75% of the Hg present in the biosphere. The highest Hg concentrations are usually found in the uppermost soil layers mainly due to Hg affinity for soil organic matter. However, it was also reported the presence of large concentrations of Hg in deeper soils layers, as occurs in podzols where podzonic horizons (Bs, Bhs, Bs) become the last barrier against the mobilization of Hg towards groundwater and surface waters, which could increase notably the risk of environmental toxicity. The immobilization of Hg in podzol B horizons depends on the interactions between Hg and soil organic matter, metal-humus complexes and Al and Fe oxyhydroxides, decreasing the risk associated to soil aggregate size fractions. This study is focused in the determination of total Hg (HgT) in coarse sand, fine sand, coarse silt, fine silt and clay fractions of five B horizons of sub-Antarctic forest soils from Tierra del Fuego (Argentina). The total Hg content in the fine earth fraction (< 2 mm) ranged between 33 and 98 µg kg⁻¹, whereas the clay size fraction showed the highest average value of total Hg (140 µg kg⁻¹; range 94-183 µg kg⁻¹) followed by the fine silt fraction which had a range of Hg₂ between 45 and 109 µg kg⁻¹. In spite of this, clay and fine silt fractions only account on average for 32% of all Hg, mainly due to the low amount of these aggregate soil fractions in the studied horizons. On the contrary, both sand fractions showed a moderate content of Hg₂, with means of 79 and 84 µg kg⁻¹ for coarse and fine sand, respectively. This implies that 56% of Hg in these soils is associated to soil aggregates of sand and silt size, and this mobilization will be easier under erosion events that could destroy the uppermost A and E horizons, leaving exposed the Bs horizons. This process can be expected in forest soils that undergo changes in its land use such us logging activities, which are spreading rapidly in some areas of Tierra del Fuego. However, in the podzonic horizons HgT seems to be strongly bound to organic C, metal-humus complexes and Al and Fe oxyhydroxides, decreasing the risk associated to soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions. This study is focused in the discrimination of related soil aggregate size fractions.
However in the assessment of Hg toxicity in soils, total Hg (HgT) content is often used as a key parameter leaving aside its distinctive origin. The origin of Hg, discriminating between atmospheric Hg (HgAtm) and lithogenic Hg (HgLith.), was assessed in six podzols from Tierra del Fuego (Argentina). Lithogenic Hg was estimated as: [HgLith] = [Ti]_TOT × ([Hg]/[Ti])_Elutra where Ti is a conservative element not influenced by atmospheric sources, soil refers to each soil layer and [Hg]/[Ti] is the lowest in E horizon and the highest in C horizon. Atmospheric Hg is calculated as: [HgAtm] = [HgT] - [HgLith]. The brackets indicate the concentration of Ti (g kg⁻¹) and Hg (µg kg⁻¹). The mean HgLith values range between 137 and 49 µg kg⁻¹ in O and A horizons, respectively. In O horizons, HgT comes mostly from atmospheric sources (HgAtm = 94% of HgT), which is reduced in A horizons. The studied soils have a background Hg content (without Hg settling and C. Schanning et al. (2006) for 3 µg kg⁻¹), presumably all from lithogenic origin. It was noticeable that spodic horizons (Bhs/Bs), which are subsurface horizons, showed the second largest HgT concentrations (average 52 µg kg⁻¹; range 15-97 µg kg⁻¹) of which up to 33% of HgT derived from atmospheric sources. This involves the mobilization of part of the Hg deposited in uppermost soil layers due to pedogenic processes (podzolization), which is consistent with the low Hg2⁺ concentration (mean: 21 µg kg⁻¹) and the high proportion of Hg₂+_T (95% of HgT) found in E horizons. The presence of these subsurface soil horizons enriched in HgAtm, such as in podzols, constitute an effective barrier against the transport of Hg to water courses, minimizing the risks of environmental toxicity associated to Hg release to groundwater and surface waters.

TU168 Ecotoxicological risks of soils surrounding an abandoned mercury mine

J. Bogli, Universitat Politècnica de Catalunya (UPC) / Centre for Research and Innovation in Toxicology CRITT; B. Valles, Universitat Politècnica de Catalunya UPC / Centre for Research and Innovation in Toxicology CRITT; A. Navarro, M. Riva Juan, Universitat Politècnica de Catalunya

The abandonment of mercury mining areas is generally associated with a release of Hg into the environment. Due to its potential toxic properties, the mobilization of particulate and soluble Hg species is of major concern in areas surrounding Hg mines. The transport of mercury from soil is mainly caused by wind action, sediment transport, water leaching or atmospheric emissions. Once in the aquatic compartment, interactions with the aqueous phase and with associated solid media will influence the mobilization of mercury. In the present study, a representative number of soil samples containing mining wastes from an abandoned mining area in the Valle del Aguqé (Almería, Spain) were assessed to study the potential risk that their presence represent for terrestrial and aquatic organisms. Samples were collected by the UAM regional authority according to the Hg levels showing a total mercury concentration in soils and wastes comprised between Eisenia fetida and earthworms from the species Folsomia candida. The toxicity to the aquatic compartment was assessed after leaching the sampled soils and through the growth inhibition test of Pseudokircheriella subcapitata and the lethality test to Daphnia magna.

TU169 Transcriptome analysis of the earthworm Eisenia fetida exposed to mercury-contaminated soils collected in French Guiana using RNA-sequencing approach

C. COCQUERELLE, PRES Lille University North of France / LGCQ-E, University of Lille; N. Bousserrine, Université Paris C. COCQUERELLE

In the assessment of Hg toxicity in soils, total Hg (HgT) content is often used as a key parameter leaving aside its distinctive origin. The origin of Hg, discriminating between atmospheric Hg (HgAtm) and lithogenic Hg (HgLith), was assessed in six podzols from Tierra del Fuego (Argentina). Lithogenic Hg was estimated as: [HgLith] = [Ti]_TOT × ([Hg]/[Ti])_Elutra where Ti is a conservative element not influenced by atmospheric sources, soil refers to each soil layer and [Hg]/[Ti] is the lowest in E horizon and the highest in C horizon. Atmospheric Hg is calculated as: [HgAtm] = [HgT] - [HgLith]. The brackets indicate the concentration of Ti (g kg⁻¹) and Hg (µg kg⁻¹). The mean HgLith values range between 137 and 49 µg kg⁻¹ in O and A horizons, respectively. In O horizons, HgT comes mostly from atmospheric sources (HgAtm = 94% of HgT), which is reduced in A horizons. The studied soils have a background Hg content (without Hg settling and C. Schanning et al. (2006) for 3 µg kg⁻¹), presumably all from lithogenic origin. It was noticeable that spodic horizons (Bhs/Bs), which are subsurface horizons, showed the second largest HgT concentrations (average 52 µg kg⁻¹; range 15-97 µg kg⁻¹) of which up to 33% of HgT derived from atmospheric sources. This involves the mobilization of part of the Hg deposited in uppermost soil layers due to pedogenic processes (podzolization), which is consistent with the low Hg²⁺ concentration (mean: 21 µg kg⁻¹) and the high proportion of Hg₂+_T (95% of HgT) found in E horizons. The presence of these subsurface soil horizons enriched in HgAtm, such as in podzols, constitute an effective barrier against the transport of Hg to water courses, minimizing the risks of environmental toxicity associated to Hg release to groundwater and surface waters.

TU170 Enhanced relative methyl mercury concentrations within a valuable seagrass area in the braackish fjord Gunneklevfjorden, Norway

M. Olsen, F. Moy, Institute of Marine Research; E. Lydersen, Telemark University College; M. Schanning et al. (2006) for 3 µg kg⁻¹), presumably all from lithogenic origin. It was noticeable that spodic horizons (Bhs/Bs), which are subsurface horizons, showed the second largest HgT concentrations (average 52 µg kg⁻¹; range 15-97 µg kg⁻¹) of which up to 33% of HgT derived from atmospheric sources. This involves the mobilization of part of the Hg deposited in uppermost soil layers due to pedogenic processes (podzolization), which is consistent with the low Hg²⁺ concentration (mean: 21 µg kg⁻¹) and the high proportion of Hg₂+_T (95% of HgT) found in E horizons. The presence of these subsurface soil horizons enriched in HgAtm, such as in podzols, constitute an effective barrier against the transport of Hg to water courses, minimizing the risks of environmental toxicity associated to Hg release to groundwater and surface waters.

The mercury concentration, enrichment and inventory in core sediments from an urban water supply reservoir in NW Spain

P.P. Rodriguez, Universidade de Vigo / Plant Biology and Soil Science; S. Peña-Rodriguez, University of Vigo; A. Gómez-Armento, University of Vigo / Plant Biology and Soil Science; L. Cutillas-Barreiro, University of Vigo; P. Araujo-Nespeira, University of Vigo / Geotecnia and Hydrology; J. Gómez-Muñoz, University of Vigo

Mercury is a global contaminant which is mainly stored in soils, but changes in land use (reforestation, agriculture, etc.) and forest fires facilitate its transport to water courses, minimizing the risks of environmental toxicity associated to Hg release to groundwater and surface waters.
Mercury bioaccumulation and transformation processes.

TU174

Mechanisms of Hg accumulation in the aquatic plant Elodea nuttallii
N. Regier, University of Geneva / Institute Forel Earth and Environmental Sciences; C. Cosio, Geneva University / Institute Forel Earth and Environmental Sciences;

Biomagnification of Hg in food webs is still a big concern nowadays in aquatic ecosystems. In shallow waters the highly toxic methyl-Hg (MeHg) is formed and aquatic plants are the predominant primary producers playing a key role in Hg transfer in food webs. Mechanisms of Hg accumulation are still unclear with earlier data suggesting passive uptake, while few recent data suggest carrier mediated accumulation. The present study aimed to elucidate the role of plant uptake and bioaccumulation in a representative aquatic plant, Elodea nuttallii. High Hg tolerance and accumulation were observed in E. nuttallii. Competition with Cu resulted in a significant 97.2±0.6% inhibition of Hg uptake while other metals (Fe, Mg, Mn, Ca, and Zn) had no significant effects on accumulation, supporting a putative role of Cu transporters in Hg uptake. RNAseq and RT-qPCR analysis further revealed a correlation between the level of expression of EnCOPT1 gene and Hg exposure. As COPT1 is known to transport Cu from the surrounding medium inside cells in different organisms, we further tested the function of COPT1 in Hg accumulation in yeast and Arabidopsis thaliana. Analyses of EnCOPT1 gene in yeast confirmed its ability to uptake Cu and confer tolerance to Hg. Lines of A. thaliana deficient and overexpressing COPT1 showed a clear correlation between the expression of EnCOPT1 and accumulation of Hg. Results supports the hypothesis that Hg is accumulated through the Cu homeostasis network in plants, further suggesting that Hg accumulation is carrier mediated, and not passive in aquatic plants. Mechanistic understanding of bioaccumulation of Hg in primary producers will strongly contribute to environmental risk assessment of Hg and will help environmental managers to take appropriate decisions to limit Hg transfer in food webs.

TU175

Mercury impacts towards periphytic communities
S. Le Faucheur, Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences; A. Freiburghaus, University of Geneva / Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences; V.I. Slaveykova, University of Geneva / Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences;

Mercury impacts towards periphytic communities are crucial to better understand the interactions between Hg and aquatic ecosystems. They have important roles in aquatic food chains as primary producers and in trace element cycling, notably mercury (Hg), which is known to be absorbed and transformed by periphytic microbes. In contrast, Hg effects towards biofilms are poorly known. The present study thus aimed to assess Hg impacts on periphyton and their sensitivity to Hg. To that end, periphyton were grown under controlled conditions using flow-through microcosms containing Geneva Lake water. Three of them were spiked with 20 pM, 200 pM and 2 nM Hg\textsuperscript{II} whereas one was not contaminated and used as a control. After 7 weeks of colonisation, periphyton were analysed for their total Hg content and strongly bound Hg content (Hg\textsubscript{SS}, i.e. after a cysteine washing step). Their chlorophyll a content and ash free dry mass (AFDM) were also measured along with their abiotic and biotic fractions using microscopy and taxonomic composition using pyrosequencing. Modification of their sensitivity to Hg was assessed by further exposing grown periphyton to 2 nM Hg\textsuperscript{II} for 24 h and evaluating damage on microbial membrane permeability using propidium iodide (PI) and cellular oxidative stress using CellRox® green. Total Hg content in control periphyton was found to be 0.33 ± 0.01 nmol/g dw and to increase by two times upon exposure to 200 pM Hg. Strongly bound Hg was also observed to increase, from 0.13 nmol/g dw in control periphyton to 0.47 nmol/g dw in periphyton exposed to 200 pM, representing 64% of the total content. At the highest Hg concentration, Hg\textsubscript{SS} represented only 10% of the total Hg content, suggesting that Hg bound to weaker binding sites at very high Hg concentrations. Modification of periphyton composition could not be observed with chlorophyll a content and AFDM modified water and deosmosis revealed a decrease of the biotic fraction in periphyton exposed to 2 nM Hg\textsuperscript{II}. Taxonomic analysis further revealed a shift in microbial communities from algal dominated (87%) to bacteria dominated communities (69% at 2 nM) with exposure to Hg. PI fluorescence was similar in control and Hg exposed periphyton. However, cellRox® green signal was lower in control periphyton than in Hg pre-exposed periphyton, suggesting that oxidative stress was higher in pre-exposed biofilms. The present study demonstrated the strong impacts of Hg on periphyton.

TU176

Mercury bioaccumulation and effects on the transcriptome in the macrophyte Elodea nuttallii
R. Beauvais-Flueck, Institute Forel Earth and Environmental Sciences; V.I. Slaveykova, University of Geneva / Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences; C. Cosio, Geneva University / Institute Forel Earth and Environmental Sciences;

Mercury is one of the most harmful pollutants which can lead to important risk for waterlogging and high in organic matter). The potential transformation of Hg in methyl-Hg in the studied reservoir deserves a detailed investigation as soon as possible at the light that, although after a careful purification, the water dammed is used for drinking.

TU172

A regional analysis of the distribution of mercury in the aquatic ecosystem of the Limpopo Watershed, South Africa
V. Somerset, NRE; M. Cuoto, Eskom; C. Walters, CSIR / Natural Resources and the Environment

In South Africa the magnitude of Hg pollution was relatively low on the agenda in 2000, although a study by Pacina et al. [1] has ranked the country as the second highest anthropogenic emitter of Hg. This data was largely based on the Hg emissions from coal combustion and artisanal gold mining, reporting that South Africa contributes more than 10% to the global Hg emission budget. South African Scientists have since then conducted further investigations and have reported that Hg emissions are significantly lower than previously reported [2]. The primary objective of the current study is to identify the main sources and transport mechanisms of Hg in the Limpopo Water Management Area (WMA), to establish a mass-balance for Hg and determine the fluxes between various ecosystem compartments. The Waterberg region is situated in this WMA and houses the building of the Medupi Power Station near Lephalale, which is the biggest dry-cooled coal-fired power station in South Africa and the world [3]. Various sources of site specific data for the Waterberg region in the Limpopo WMA have been gathered that includes Hg concentrations in water, sediment, periphyton. New data builds on the findings by Walters et al. [4] in which the Hg concentrations for the Limpopo WMA was identified to be: Tothg < 10 ng/L and MeHg < 1 ng/L in freshwater; while in sediment the Tothg < 10 ng/g and MeHg < 1.00 ng/g. The pathway for the wet and dry deposition of Hg is also investigated and an assessment of the roles of seasonal variability, wind direction and precipitation are analysed. Analysis of the data involves the fluxes of Hg across the sediment–water interface, water-air interface and water-biota transfer. Furthermore, the regional Hg budget and the factors contributing to major uncertainties associated with Hg emission estimates and Hg chemistry are also evaluated. It is believed that once the new dry-cooled coal-fired power station is in operation, changes in atmospheric Hg deposition to the aquatic ecosystems will occur, resulting in flux changes of Hg across the sediment–water interface, water-air interface and water-biota transfer. References: [1] Pacyna, E.G., et al. (2006). Atmos. Environ. 40,4048-4063.[2] Dabrowski, J.M., et al. (2008). Atmos. Environ. 42 6620-6626.[3] http://www.eskom.co.za/Whatweredoing/NewBuild/MedupiPowerStation/Pages/[4] Walters, C.R., et al. (2011). J. Environ. Sci. & Health A46,1129–1137.

TU173

Kinetics of mercury accumulation and transformation in periphyton
P. Dranget, Institut Forel, University of Geneva / Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences; S. Le Faucheur, Section of Earth and Environmental Sciences Institute Forel and Institute for Environmental Sciences;

Periphyton is a molten pot containing a community (micro)organisms and abiotic fraction (organic matter, polysaccharide matrix, algae) essential for the proper functioning of aquatic ecosystems. Mercury (Hg) pollution in the environment is dangerous and presents a threat to human health. The aim of the present study was to examine the kinetics of Hg accumulation and transformation by periphyton and to evaluate the role of periphyton composition in these processes. To that end, periphyton was cultured on microscopy slides with a flow-through microcosm containing Geneva Lake water during 85 days and 127 days. Formed biofilms were then exposed to 200 pM Hg during 10, 30 min, 1, 4, 6, 8 and 24 hours and examined for Hg content for each time. The composition of the two communities was analysed using pyrosequencing. Total Hg and non-extractable Hg\textsuperscript{II} and CH\textsubscript{Hg} (Hg obtained after cysteine washing) accumulation were measured after acid digestion by cold vapour atomic fluorescence spectrometry. Physicochemical parameters of exposure media (pH, temperature, cations and dissolved organic matter concentrations) as well as Hg concentrations were also analysed at each exposure time. Total accumulated Hg in periphyton were comparable for both communities in the first hours of exposure. However, after 24h, these concentrations were 886±179 pM/g dry weight for the youngest periphyton and 152±873 pM/g dry weight for the oldest periphyton. Non-extractable Hg contents were similar in both communities. The percentage of Hg strongly attached to periphyton represents thus around 48% for the 85 days periphyton and 34% for the 127 days periphyton. Moreover, chlorophyll a content decreased with increasing Hg accumulation, suggesting an impact on photosynthesis process. Further analysis on periphyton composition will allow us to better understand the influence of periphyton composition on biocaccumulation and transformation processes.
the environment at global scale. Moreover Hg exists in diverse forms, both inorganic (IHg) and organic, like methyl mercury (MeHg). Inorganic Hg and MeHg are very toxic and known to accumulate in primary producers, but MeHg is further biomagnified in the aquatic food chain, resulting in health hazard for higher consumers. Elodea nuttallii is a submerged plant rooted in sediments. Like other primary producers it plays an important role in Hg biogeochemical cycle in freshwater systems. Previous study on Hg accumulation in E. nuttallii highlighted high Hg concentration but differences in uptake kinetics and sub-cellular distribution of IHg and MeHg, and thus the possibility that these two Hg forms are taken up by different pathways or are regulated by different mechanisms in primary producers. The objective of our study was thus to assess the effect of Hg internalization on the level of gene expression using high-throughput sequencing with the aim of understanding the mechanisms behind Hg accumulation and toxicity. E. nuttallii was exposed two hours in a simplified artificial medium to increasing concentrations of 1.0 × 10⁻⁸ to 1.0 × 10⁻⁴ M IHg or 4.6 × 10⁻⁸ to 4.6 × 10⁻⁴ M MeHg. Although the tested concentrations do not have significant effects on photosynthesis, oxidative stress, or growth, the analysis of the transcriptome data obtained by RNA-Seq enables us to investigate the molecular mechanisms of the response of this plant to Hg and to find interesting genes that can be used as biomarkers for Hg and/or MeHg exposure in the aquatic environment.


Mercury was used in many industrial and agricultural applications in the past. In the UK today most deliberate uses have ceased, but it is still used in dentistry and some electronic components (mainly fluorescent lights), and it is also released as a trace component of fossil fuels. The UK fish tissue archive Fish Fossil Archive is a tool for retrospective monitoring of pollutants. We analysed a total of 144 roach and bleak (whole body homogenates) from the UK Fish Tissue Archive for mercury and found: Overall the mercury concentrations were in a relatively narrow range between 6 and 68 µg/kg with more than 3/4 of the values above the EU Environmental Quality Standard of 20 µg/kg fresh weight, but well below the food standard of 500 µg/kg. Mercury concentration tended to increase with the distance from the fish and was overall a little higher in bleak than in roach. After adjusting for fish size, differences became apparent: In the upper reaches of rivers there was an increase of size-adjusted mercury concentration with distance from the source, but this increase did not continue in the lower reaches (lower reaches only available for the Thames). One explanation for this may be less favourable conditions for methyl-mercury transformation in the upper reaches. Results indicate that in the Thames, London. The Thames, London has the highest mercury (Hg(II)) and methylmercury (MeHg(II)) levels of 3.39 × 10⁻⁴ to 1.12 × 10⁻³ mg L⁻¹ and 2.042 and 0.079 mg kg⁻¹ ww in water and sediment, respectively. Wherever in northern Taiwan, Tamsui River had the highest Hg level. Based on the established PBTK model, the highest and lowest tissue/organ burdens in Tilapia in Laemen Greek were found in kidney and muscle, respectively, of 0.135 and 3.301 µg g⁻¹ tissue, respectively. Results indicated that muscle had the lowest total body half lethal dose (LD₅₀) of 2.31 and 2.88 kg (mean ± SD) µg g⁻¹ ww (r² = 0.94, p < 0.001), whereas liver had the highest LD₅₀ of 7.15 ± 9.10 µg g⁻¹ ww (r² = 0.93, p < 0.001). The likely mortality risks were estimated to be 5.668% (95% CI: 0 – 35.060), 5.500 (0 – 50.278), and 5.500 (0 – 48.924) in gill, liver, and muscle, respectively. This study provides an integrated risk assessment framework to better assess the environmental impact of mercury/methylmercury exposure on mortality of tilapia species based on an integrated approach that links a water-sediment dynamic model, a PBTK model, and a probabilistic risk model.

TU181 Sub-cellular partitioning of mercury in the liver of wild golden grey mullets (Liza aurata) - a case study of ria de Aveiro (Portugal) O. Araujo, DIVOIA; P. Pereira, Department of Biology and CESAM; M. Pacheco, University of Aveiro / Dept of Biology; J. Raimundo, IPMA / DIVOIA 

Mercury (Hg) has been identified as a severe environmental pollutant to the aquatic biota. However, little is known about its sub-cellular partition in wild fish. Thus, the current study aimed to determine sub-cellular partitioning of mercury (Hg) in the wild and sub-cellular fractions of golden grey mullet (Liza aurata), namely granules (Gran), mitochondria (Mit), nuclei plus cellular debris (N+D), lysosomes plus microsomes (Lys+Mic), and then analyze their relationship with growth, oxidative stress, or liver pathology. The results indicated that Hg was more concentrated in the mitochondrial fractions of golden grey mullet, namely granules (Gran), mitochondria (Mit), nuclei plus cellular debris (N+D), lysosomes plus microsomes (Lys+Mic), and then analyzed their relationship with growth, oxidative stress, or liver pathology.

TU179 Mercury in the big-scale sand smelt (Atherina boyeri Risso, 1810) of the Marano and Grado Lagoon (northern Adriatic Sea) A. Bettolin, A. Felluga, G. Piazza, E. Rancati, A. D’Asetti, L. Faresi, G. Mattassi, ARPA FVG

The big-scale sand smelt, Atherina boyeri (Risso, 1810), is a small, short-lived, euryhaline atherinid fish which mainly inhabits coastal and estuarine waters, including coastal lagoons, salt marshes and, more rarely, inland waters, over a wide range of salinities from freshwater to hypersaline conditions. It is common in the Mediterranean Sea and, although occurring in the western part of the Azores to the North-West coast of Scotland. A. boyeri represents one of the most important fisheries resource in the Marano and Grado Lagoon, being caught by fyke nets, a traditional fishing gear in the northern Adriatic lagoons. The Marano and Grado Lagoon experienced an historical mercury (Hg) contamination due to both mining and chlor-alkaly activities. As a consequence this environment suffers from a diffuse sediment contamination, which is actively transferred to the related trophic chain. The aim of this study was to investigate the total Hg concentration in the edible tissue of the sand smelt during spring 2012. Specimens were caught in 22 sampling stations and stored at -20°C until the analysis. On each station 100 individuals were randomly selected and the total length (TL) was recorded. 40 g of fishes was homogenized for each sample and Hg analysis was performed using the EPA method 7477H. Mean TL of sand smelts in the sampling stations ranged from 6.26±0.86 cm to 8.42±0.79 cm (mean value 7.29 cm), whereas Hg concentration ranged from 0.28 µg g⁻¹ ww (wet weight) to 0.85 µg g⁻¹ ww (mean value 0.52±0.18 µg g⁻¹ ww). These preliminary data showed the level of Hg concentration in this species, caught during the breeding period. Further investigations are clearly required in order to assess Hg levels during other seasons and to verify the existence of a spatial gradient of bioconcentration in the Marano and Grado Lagoon.

TU180 PBTK/TD model-based mortality risk assessment for tilapia species exposed to mercury/methylmercury Y. Cheng, Y. Lin, Y. Shu-Han, National Taiwan University / Department of Bioenvironmental Systems Engineering; Y. Yang, National Taiwan University; C. How, Y. Tseng, V. Liao, C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering

Environmental mercury (Hg) is considered one of the most hazardous chemicals due to the documented adverse effects on aquatic organisms. Therefore, the overall objective was fourfold: (i) to quantify environmental Hg levels in six rivers across northern and southern Taiwan, (ii) to estimate tissue burdens in tilapia species exposed to mercury/methylmercury (Hg(II)) and methylmercury (MeHg(II)) levels of 3.39 × 10⁻⁴ to 1.12 × 10⁻³ mg L⁻¹ and 2.042 and 0.079 mg kg⁻¹ ww in water and sediment, respectively. In northern Taiwan, Tamsui River had the highest Hg level. Based on the established PBTK model, the highest and lowest tissue/organ burdens in Tilapia in Laemen Greek were found in kidney and muscle, respectively, of 0.135 and 3.301 µg g⁻¹ tissue, respectively. The PBTK model revealed that muscle had the lowest total body half lethal dose (LD₅₀) of 2.31 and 2.88 kg (mean ± SD) µg g⁻¹ ww (r² = 0.94, p < 0.001), whereas liver had the highest LD₅₀ of 7.15 ± 9.10 µg g⁻¹ ww (r² = 0.93, p < 0.001). The likely mortality risks were estimated to be 5.668% (95% CI: 0 – 35.060), 5.500 (0 – 50.278), and 5.500 (0 – 48.924) in gill, liver, and muscle, respectively. This study provides an integrated risk assessment framework to better assess the environmental impact of mercury/methylmercury exposure on mortality of tilapia species based on an integrated approach that links a water-sediment dynamic model, a PBTK model, and a probabilistic risk model.

TU178 Mercury Concentrations in Freshwater Fishes on North Pacific Islands: the Influence of Military Sites, Atmospheric Deposition & Biotransport P.E. von Hille, University of Alaska Anchorage / Department of Biological Sciences; L.A. Kenney, Alaska Natural Heritage Program / Biological Sciences; C. Eagles-Smith, FRESC; J. Ackerman, USGS / Western Ecological Research Center; R. Kaler, US Fish & Wildlife Service / Migratory Bird Management

Some islands in the Aleutian Archipelago and Gulf of Alaska have been heavily impacted by military activity and accidental release of mercury (Hg) into the environment. This study was designed to determine long-range contaminant transport via atmospheric distillation, precipitating ocean currents, and biotransport. We evaluated mercury concentrations of 1,121 resident-freshwater fish samples of two species, the three-spine stickleback (Gasterosteus aculeatus) and Dolly Varden (Salvelinus malma). Samples were obtained across a longitudinal gradient of 11 islands spanning over 3,000km and including marine bird lakes. Mercury concentrations were higher throughout our sampled islands than in lakes in mainland Alaska. At all islands, mean fish mercury levels exceeded Environment Canada’s advisory level for fish-eating wildlife (0.033 mg kg⁻¹ ww). These results provide the first mercury levels in resident-freshwater fishes across the Aleutian Archipelago, a area experiencing effects of rapid climate change. The results also provide critical baseline data for wildlife management and human consumption advisories.
Monitoring and modelling a neurotoxin, our analyses focus on neurotoxic modes of action. Moreover, levels in Mit, Gran and HDP were linearly correlated with Hg concentrations in the water column. Differences in Hg concentrations may be discerned for Lys+Mic and whole liver. This could be indicative that a certain threshold of accumulation was exceeded. The association of Hg to metal sensitive fractions (e.g. Mic, Mit and HDP) suggests that the metal detoxification mechanisms were not efficient in liver of L. aurata living under chronic exposure conditions.

TU182 Temporal and spatial assessment of mercury in fish of the Madeira River, Brazil

W.R. Bastos, Universidade Federal do Rio Grande do Sul; J.G. Dorea, Universidade de Brasília / Nanotechnology; B. Furtado, Universidade de Brasília / Nanotechnology; L.L. Lauter, M. Mussuy, Federal University of Rondônia; L. Lacerda, Federal University of Ceará; O. Malm, Fed Univ Rio de Janeiro / Biophysics Institute

The Madeira River is the tributary of the Amazon River Basin and one of the most impacted by artisanal gold-mining activities, deforestation for agricultural projects, and recent hydroelectric reservoirs. We studied total Hg (and methyl-Hg) concentrations in 3,211 fish samples of 84 species (3 genders) of different trophic levels as a function of standard size. Species at the top of the trophic level (Piscivorous, Carnivorous) showed the highest mean total Hg concentrations (50.9 to 1,241.7 µg/kg), Planctivorous and Omnivorous species showed the intermediate total Hg concentrations (25.8 to 494.0 µg/kg), while Detritivorous and Herbivorous species showed the lowest range of mean total Hg concentrations (9.4 to 274.8 µg/kg). Significant differences between species were found (Welch's t-test; p<0.05) and concentrations were seen for Planctivorous (t=0.474, p=0.0001), Piscivorous (t=0.459, p=0.0001), Detritivorous (t=0.227, p=0.0001), Carnivorous (t=0.212, p=0.0001), and Herbivorous (t=0.156, p=0.01), but not for the Omnivorous species (t=0.064, p=0.0685). Moreover, feeding habit of species influenced theratio of MeHg to total Hg (ranged from 70 to 92%). When adjusted for standard body length, significant increases in Hg concentrations in the last 10 years were species specific. Spatial differences indicate that significant difference but no time trends consistent with environmental contamination of past alluvial gold mining activities. Fish-Hg bioaccumulation is species specific but fish feeding strategies is the predominant influence in the fish-Hg bioaccumulation pattern. Despite spatial differences in fish-Hg concentrations, short term temporal changes indicate an increase in Hg bioaccumulation pattern of fish species collected before impoundment of areas under the influence of Santo Antonio Dam.

TU183 Mercury in Arctic Barnacle goslings in contaminated terrestrial habitats

K. van den Brink, Wageningen University / Dept of Toxicology; I.B. Scheiber, University of Groningen / Centre for Ecological and Evolutionary Studies Behavioural Ecology and Selforganization; M.E. de Jong, University of Groningen / Arctic Center; A. Braun, University of Groningen / Centre for Ecological and Evolutionary Studies Behavioural Ecology and Selforganization; M.J. Loonen, University of Groningen / Arctic Center; T. Groot and T. Bastian

This study focuses on mercury from different sources. Long-range transport brings mercury from lower latitudes to the Arctic via the atmosphere. Local human activities may result in contamination of confined areas. For instance, mining activities have resulted in elevated levels of mercury in the surroundings of the mining areas. Research on mercury in the Arctic has mainly focussed on marine ecosystems and receptors, which are regarded as being mostly at risk. On the other hand, risks of local contamination of terrestrial ecosystems by human activities have been studied less. In the current paper we will present a pilot-study, in which we herded goslings in a mine impacted site and a control site near Ny-Ålesund, Svalbard. Herding of the goslings made it possible to perform a controlled study under field conditions, assessing the accumulation of mercury in the two groups of goslings raised in the two areas. Soil in the mining area contained 5-6 times higher concentrations of total mercury in comparison to the control site (based on soil geomorphic means). This was reflected in elevated levels of mercury in the vegetation in the mining area (approx. 2.2 times higher compared to the control site). Each group of goslings grazed approximately 3-4 hours per day in their respective areas. At night and during bad weather conditions they were kept in pens. Therefore, goslings in the mine impacted site showed an increased intake of total mercury in the liver relative to control goslings (p < 0.05). Concentrations were relatively low, possibly due to growth dilution and deposition of mercury in the growing feathers. All goslings received supplemental food, hence effects of mercury on growth rate could not be assessed. Currently, biochemical analyses are underway in order to determine effects at the sub-individual level. Since mercury is a neurotoxin, our analyses focus on neurotoxic modes of action. Additionally, we exposed goslings to moderate stressors, and observed their behaviours. Combined results will be presented at the meeting.

Monitoring and modelling-based approaches for identification and prioritisation of hazardous emerging pollutants in European freshwater resources (P)

TU184 Impact of multi-stress on macrophyte vegetation in the Danube Basin: the experience from the Joint Danube Surveys (JDS 2 & 3)

M. Novkovic, D. Cvijanovic, S. Radulovic, University of Novi Sad Faculty of Sciences / Department of Biology and Ecology; I. Teodorovic, University of Novi Sad

The aim of this study, undertaken within the frame of collaborative FP 7 project Solutions, was to select the most important stressors on macrophyte vegetation assemblages’ diversity along the Danube, based on the Joint Danube Survey 2/3 databases. The influence of land use types, basic physico-chemical parameters and pesticides were tested against i) vegetation structure, ii) macrophyte richness and iii) macrophyte production. Vegetation data matrix was compiled using the macrophyte functional groups. Canonical Correspondence Analysis (CCA) was performed in order to assess the impact of multiple environmental stressors on vegetation structure. All ordination-associated analyses were performed with the CANOCO 4.5 software package. Macrophyte richness and production were correlated with environmental variables using the Statistica 12. Macrophyte richness and production were moderately influenced by presence of wetlands and artificial land cover types in the riparian zone. In opposite, results of the CCA analysis revealed that the land use parameters do not influence vegetation structure directly. Basic water quality parameters and selected organic pollutants (Bentazon, Bisphefalon A, Desethyl-atrazine, Diolofenac, Ispropfen, Ketoprofen, Simazine), explained together approximately 35% of total variance of vegetation structure, while selected pollutants explained over 28 % of total variance. The SOLUTIONS project is supported by the Seventh Framework Programme (FP7-ENV-2013) of the European Union under grant agreement no. 603437.

TU185 Discriminating between the effects of chemicals and other influencing factors on the macroinvertebrate community and trait composition: data analysis from the 3rd Joint Danube Survey

A. Focks, Alterra Wageningen University and Research Centre / Ecotoxicology Environmental Risk Assessment Team; W. Graf, BOKU - University of Natural Resources and Life Sciences Vienna / Institute for Hydrobiology Water Management; A. Schmidt-Kloiber, P. Leitner, BOKU - University of Natural Resources and Life Sciences Vienna; I. Teodorovic, University of Novi Sad; M. Paunovic, IBISS; P.J. van den Brink, Alterra and Wageningen University

In large rivers such as the Danube, a number of different factors can affect the composition of aquatic macroinvertebrate communities: hydromorphological conditions, nutrient levels, general water quality parameters such as SOM, pH or salinity, but also organic chemicals or metals. A prerequisite for an assessment of such influences is having enough data at hands with most important factors included to ensure robust statistical analyses. In the scope of the 3rd Joint Danube Survey (JDS3), for 68 sampling sites across the entire length of the Danube River an extensive dataset was collected. The data collection was organised by the International Commission for the Danube River (ICPDR) together with all 14 main Danube Basin countries and the European Commission. The data consists of hydromorphological parameters, water quality parameters and results from chemical target analysis for more than 300 organic chemicals and metals. In addition, data was collected for several biological groups, e.g. macroinvertebrates, fish or aquatic macrophytes. For our analysis we focused on the assessment of the taxonomic composition of the macroinvertebrate community. Abundances of macroinvertebrate species were sampled via kick & sweep method and microhabitat sampling techniques. The resulting community composition data were transformed into trait composition by using species-trait tables, amongst other sources from the freshwaterecology.info database. Multivariate statistical techniques were applied to determine the influence of the above mentioned water parameters on the taxonomic and functional composition of the invertebrate community with the objective to identify those factors that explain variations across the sampling sites. Further, we wanted to filter for the influences of different categories of explaining factors. Hence we applied the technique of variance partitioning, which can be utilised to partition explained variability in the data into a number of categories. As expected, the variability in the invertebrate composition was co-determined by quite a number of factors form different categories, and again hydromorphology was found to be most influential. Nevertheless, it was possible to identify the most influential chemical compounds as well. Acknowledgement: The SOLUTIONS project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 603437.

TU186 Cultivated-Based Approaches in Analysis of Fish Communities Help in Identification of Key Pollutants and Mixtures: The River Danube Case Study

S. Sipos, Faculty of Sciences / Leocros. Laboratory of Ecotoxicology; V. Jovanovic, Institute for Biological Research Siniša Stanković University of Belgrade; V. Bammer, Austrian Federal Agency for Water Management; L. Sladkic, Institute for Biological Research Siniša Stanković University of Belgrade; V. Bammer, Austrian Federal Agency for Water Management; L. Sadanic, Institute for Biological Research Siniša Stanković University of Belgrade; V. Bammer, Austrian Federal Agency for Water Management;
Pehilvanov, Bulgarian Academy of Sciences / Institute of Biodiversity and Ecosystem Research; H. Zornig, M. Schabus, PRO FISCH OG C. Szalayko, Danube Research Institute / MTA Centre for Ecological Research; A. Focks, Alterra Wageningen University and Research Centre / Ecotoxicology Environmental Risk Assessment Teams; I. Teodorovic, University of Novi Sad Species traits provide a reliable means to understand general rules in community ecology, along environmental or landscape gradients, as the river continuum concept suggests. However, these hypotheses are mainly based on the longitudinal gradients in habitat structure and productivity. Trait-based approaches are gradually replacing taxonomy-based approach in aquatic ecosystem status assessments, but mainly to study responses of fish species and communities to hydromorphological alterations and processes of fish species extinction, invasion and assemblage homogenization. Hydromorphological alterations, eutrophication and biological invasions for sure present key pressures to fish communities and overall ecological status of aquatic ecosystems, while adverse impact of chemical pollution on ecosystem integrity might remain hidden. The aim of the present study is to apply community composition and trait based approaches to detect and possibly quantify in situ ecological impacts of pollutants and their mixtures on fish communities and hopefully contribute to the process of identification of key or river basin specific pollutants and mixtures. The study is based on comprehensive monitoring dataset, acquired during Joint Danube Survey 3 (JDS 3) in 2013, which includes biological (focus on fish), hydromorphological and large chemical dataset (over 300 chemicals). Fish data were collected by ichthyological team of JDS 3 at 32 sampling sites along the Danube, from Beljum (DE) to Sulinia (RO). Altogether, 67 species belonging to 17 families were sampled. YG104v, an end-point genotoxic assay, is more sensitive than its parental TA98 to nitro compounds and aromatic amines. Similarly, YG5185 is more sensitive to benzo(a)pyrene like substances in comparison to TA1538. The objective of this study was to evaluate the mutagenicity profile of surface water organic extracts from the Danube campaign (JDS3) collected in 2014. High volume water samples were collected and extracted using large volume solid phase extraction (SPME) and metalloproteinase chips were used to simulate the membrane attack of the organisms to treat water samples from small catchments in areas with heavy agricultural activity. The water may be contaminated by anthropogenic chemicals and is an issue concerning a wide range of aspects from ecotoxicology to drinking water production. Many waterworks rely either directly on surface water as a raw water resource, use river water for artificial groundwater recharge or abstract bank filtrate. To provide a scientific basis for legislation and regulation and to give a snapshot of the state of water quality in the Danube River Basin, Joint Danube Survey (JDS) campaigns are held every 6 years. This study presents data of organic trace pollutants analysed in water samples taken in a longitudinal Danube profile during JDS 3 in the year 2013 and from additional bank filtrate and groundwater samples. A set of 49 compounds was analysed which comprised benzoazoles, artificial sweeteners, pharmaceuticals, iodinated X-ray contrast media, the stimulant caffeine and others. Elevated concentrations of biodegradable substances like caffeine and saccharine demonstrated the release of untreated domestic waste water, especially in Danube tributaries in the middle and lower catchment. A number of less biodegradable or rather persistent substances were detected in the Danube, its tributaries and even in bank filtrate and groundwater. Among these, the artificial sweetener aspartame had the highest concentrations (up to 2.9 µg/L). Further important pollutants were the industrial chemical 1,2-benzocarbazole and tollytriazoles (mostly below 400 ng/L), iodinated X-ray contrast media like amiodroxic acid or iopamidol (up to 1.6 µg/L) and pharmaceuticals like carbamazepine (up to 140 ng/L). Concentration levels of the trace pollutants investigated were mostly lower in the Danube than in the Rhine, most likely due to higher dilution in the Danube. Danube tributaries, especially in the middle and lower basin, were more heavily polluted both with persistent and with biodegradable compounds, pointing to discharge of untreated wastewater. The general contribution of metropel regions on deterioration of water quality was illustrated by elevated concentrations of several compounds. [1] Joint Danube Survey 3 Final Scientific Report, eds. Liska I, Wagner F, Deutsch K, Seng M, Kummrow F, Spanik M. Project 2013/16956. Solutions PRO for the Protection of the Danube River. Thanks to D. Richter, colleagues from IAWD and water suppliers. Further, thanks to JDS3 team, I.Liska and J.Slobodnik representatively.

TU189
An overview of the Danube water quality in the Novi Sad municipality during 2011-2013
N. Milic, University of Novi Sad, Faculty of Medicine / Department of Pharmacy; I. Spanik, Slovak University of Technology in Bratislava; M. Milanovic, Medical Faculty, University of Novi Sad; O. Vyvriuks, Slovak University of Technology in Bratislava; I. Mihajlovic, Borut Letic, Pharmacy; D. Milovanovic, Faculty of Technical Sciences, University of Novi Sad; M.M. Turk Sekulic, Department of Environmental Engineering and Occupational Safety and Health; J. Radonic, Faculty of Technical Sciences; M. Vojinovic MIlloradov, Novi Sad as the second largest city in Serbia faces the specific problem of the drinking water supply from several groundwater aquifers that could easily be affected by direct discharges of the municipal, industrial and agricultural activities. This research has been financially supported by NATO Science for Peace Programme (2011-2013). The main objective of the three year study was to gain more insight into the occurrence and concentration levels of emerging and priority pollutants present in the Danube surface water and wastewater of sewage discharges around the territory of Novi Sad for the Protection of the Danube River. Thanks to D. Richter, colleagues from IAWD and water suppliers. Further, thanks to JDS3 team, I.Liska and J.Slobodnik representatively.

TU187
Mutagenicity of Joint Danube Survey (JDS3) water samples using diagnostic strains of Salmonella/microsome assay
D.A. Morales, School of Technology, UNICAMP / LEAL, Laboratory of Ecotoxicology and Environmental Microtoxicology; F. Kummrow, Universidade Federal de São Paulo; M. Krauss, T. Schulze, W. Brack, HELMELHOFT Centre for Environmental Research UFZ / Effet Directed Analysis; G.d. Umbreinho, FACULTY OF TECHNOLOGY - UNICAMP / LEAL
The Salmonella/microsome assay uses strains with different metabolic capacities and DNA repair systems have proven to be useful to identify chemical classes of mutagens and target-organ specific compounds. The following 5 strains (1 TA98, 4 TA1535) were used: YG104v, YG108v, YG5185, YG5189. The strains were grown in a liquid-phenolglycol (PG) agar broth and suspensions in DMSO and assayed in the Ames assay. More than 150 organic and inorganic compounds were tested in the microsuspension protocol in dose responses experiments with a highest chemical concentration of 100 µg/mL. The results of preliminary mutagens analysis showed that the metabolic activation system is not appropriate for detecting the mutagens of the samples. The study was conducted in the framework of FP7 SOLUTIONS Project (603437).

TU185
Organic trace pollutants in the Danube Catchment - Results from Joint Danube Survey 3
E.R. Storeck, DVGW-Technologiezentrum Wasser / Analysis and Water Quality; H. Brauch, DVGW-Technologiezentrum Wasser
Pollution of surface water with anthropogenic chemicals is an issue concerning a wide range of aspects from ecotoxicology to drinking water production. Many waterworks rely either directly on surface water as a raw water resource, use river
LC-MS/MS. 87 of the analyzed pesticides were detected in different water samples. Further, the water samples were used for toxicological profiling. Oxidative stress response is a sensitive indicator of toxicity, responding to a wide variety of chemicals. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a key regulator in the cellular defense against oxidative stress through release of detoxifying enzymes. To investigate the potential of the water samples to activate the Nrf2 pathway, human liver HepG2 cells were treated with a reporter plasmid where the expression of luciferase was under the control of a promoter containing Nrf2 responsive elements. The water samples from the environmental monitoring program were extracted, concentrated and then analyzed for cytoxicity by MTS test and oxidative stress response in the transected NCI-H295R cells. No cytotoxic effects were revealed, but a large number of the samples exerted oxidative stress, even for the samples where all the detected pesticides were below the water quality standards as defined by the Swedish Chemicals Agency. In conclusion, we have established a reporter gene assay to measure oxidative stress response in concentrated water samples and detected effects in samples not displaying general cytotoxicity and with pesticide residue levels below the water quality standards.

**TU191**

**Depth profile of emerging and persistent organic pollutants, in the front of the Three Gorges Dam 2012/13**

D. Deyerling, J. Wang, Helmholtz Zentrum Muenchen / Molecular EXposomics; Y. Bi, Chinese Academy of Sciences / Institute of Hydrobiology; G. Pfister, HGMU-MEX; B. Henkelmann, Helmholtz Zentrum Muenchen; K. Sangiho and H. Byers, University of California

The Three Gorges Reservoir (China) formed by the impoundment of the Three Gorges Dam has a surface area of 1084 km². The large volume of this water body affords high efforts in terms of the analysis of organic trace compounds. Therefore a sampling method was developed in the framework of the Yangtze project, for the enrichment of large quantities of water (up to 300 L) in order to detect compounds down to the ppt level. The method was developed and refined using polycyclic aromatic hydrocarbons (PAHs), organochlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs). The method was applied twice in the middle of the Yangtze River in front of the Three Gorges Dam in 2012 and 2013. Three different water depths were sampled in order to generate a depth profile of the investigated organic trace compounds. The samples were processed by two independent cleanup methods. One method focused on a wide range of persistent organic pollutants (POPs) including polycyclic aromatic hydrocarbons (PAH), organochlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs). The other method was optimized for the analysis of compounds with higher water solubility including perfluoroalkyl acids (PFAs), active pharmaceutical ingredients and polar pesticides. POP analysis was carried out with gas chromatography and high resolution mass spectrometry. Compounds with higher polarity were analyzed with micro-scale ultra-performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer. In general, analyses seemed to occur in comparable concentrations in different water depth. Individual pharmaceutical residues and polar pesticides were detected at concentration levels between 25 and 4500 pg L⁻¹. PFAs were found in a concentration range between 0.01 and 1300 pg L⁻¹. PCBs and PAHs were mostly below the detection limit (0-400 pg L⁻¹. OCPs could be detected at trace levels between about 0 and 70 pg L⁻¹, individual PCB concentrations were in the low pg L⁻¹-scale and thus mostly in the range of blank values.

**TU192**

**Determination of auramine dye in river water samples and ecotoxicological evaluation for aquatic organisms**

C.C. Azevedo, UNICAMP; C.C. Montagner, University of Campinas UNICAMP; G.d. Umbeubece, FACULTY OF TECHNOLOGY - UNICAMP / LEAL

Auramine (CAS Number: 492-80-8) is a diphenyymethane dye used in several industrial activities, such as, textile, paper, food and leather. Auramine is probably included in nutrient rich river waters downstream of WWTP output, a known source of effluents containing the surfactants. The concentration of a broad range of surfactant including, e.g., sodium lauryl sulfate (SLS) and sodium dodecyl sulfate (SDS) has been sampled for sediment, Cladophora and phytobenthos community. The method used for this investigations was based on liquid extraction (LLE) followed by high performance liquid chromatography-diode array detector (HPLC-DAD). Acute toxicity tests were performed with Daphnia similis, and chronic tests with Ceriodaphnia dubia and Raphidocelis subcapitata. HPLC-DAD conditions were: Waters HPLC system, Shim pack VP C18 5µm, 250 x 4.6 mm, mobile phase, flow rate set at 0.8 mL min⁻¹ with detection at 437 nm. The detection limit for the auramine dye was of 0.01 µg L⁻¹ was obtained for samples that were concentration 1000 times. The recovery was 92% (±3%) for spiked river water samples (900 mL) containing 1.0 mg L⁻¹ of the dye were extracted with LLE using dichloromethane/methanol (2.5:1 v/v). EC₅₀ for D. similis was 2100 µg L⁻¹, NOEC for C. dubia, 100 µg L⁻¹ and for R. subcapitata, 20 µg L⁻¹. We conclude that the methodological approach as LLE and HPLC-DAD is sensitive and fast and is ready to be applied to real samples at quantification limits that are 2 000 times below the NOEC of the most sensitive organism tested. Real samples of effluents and water under the influence of industrial discharges are now being analyzed. The toxicity data with fish embryo test will be conducted for the derivation of a proper PNEC to be used for the comparison with the occurrence data.

**TU193**

**Systematic suspect screening and identification of sulfua drug transformation products**

M. Majewsky, Karlsruhe Institute of Technology (KIT) / Engler Bunte Institut Chemie of Water Chemistry and Water Technology; T. Glauner, Agilent Technologies / Sales Services GmbH HewlettPackardstr Waldbronn Germany; H. Horn, Karlsruhe Institute of Technology KIT / Chair of Water Chemistry and Water Technology

It has recently been shown that transformation products (TPs) of sulfamethoxazole (SMX) bearing functional groups such as hydroxylation or nitration retain significant antibacterial effects and are suspected to contribute to the development of antibiotic resistance in bacteria. Due to the homologous series of sulfonamides and their common reactive site, there is much to suggest that similar transformations occur for sulfonamide homologs other than SMX. Given that about a dozen of sulfonamide antibiotics are frequently occurring in surface waters, the screening for all transformation products using target methods would require to purchase or synthesize all potential TPs and would evidently cause unreasonably high costs and is therefore hardly realistic. For this reason and since accurate mass LC/MS/MS is not widespread in control labs, the aim of the present study was to develop a generic scheme to predict the 28 most likely TPs of a sulfonamide by identification of the major transformation reactions and pathways. The mass shifts of each transformation were used to calculate the precise ions for a targeted suspect screening with unit mass LC-MS/MS. The additional use of accurate mass LC/TOF analysis allowed for the tentative identification of TP precursors by their molecular weight and isotopic pattern. Based on the structural elucidation of the parent sulfonamide ion spectrum, mass shifts additionally allowed to predict five characteristic product ions for each derived TP. The prediction scheme was validated using polycyclic aromatic hydrocarbons (PFAAs), activic pharmaceutical ingredients and polar pesticides. POP analysis was carried out with gas chromatography and high resolution mass spectrometry. Compounds with higher polarity were analyzed with micro-scale ultra-performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer. In general, analyses seemed to occur in comparable concentrations in different water depth. Individual pharmaceutical residues and polar pesticides were detected at concentration levels between 25 and 4500 pg L⁻¹. PFAs were found in a concentration range between 0.01 and 1300 pg L⁻¹. PCBs and PAHs were mostly below the detection limit (0-400 pg L⁻¹. OCPs could be detected at trace levels between about 0 and 70 pg L⁻¹, individual PCB concentrations were in the low pg L⁻¹-scale and thus mostly in the range of blank values.

**TU194**

**The combination of sediment and Cladophora for the screening of surfactant in freshwater river.**

H. Byers, EffectDirected Analysis; L. Krauskopf, M. Kraus, Helmholtz centre for environmental research - UFZ; UEFZ; R. Jansen, UFZ; M. Schmutz-Jansen, UEFZ - Helmholtz Ctr Environm. Research / Department of Bioanalytical Ecotoxicology; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis

The wide use of surfactants in personal care product, pesticides, pharmaceutical on a global scale, makes their presence rather ubiquitious in the environment. Surfactants may be found in the aquatic environment at concentrations, which may be orders of magnitude above other micro-pollutants and result in notably high hazard quotients. Due to their amphiphilic properties surfactants are likely to sorb in a high range of biological compartments. It has recently been shown that transformation products (TPs) of sulfamethoxazole (SMX) bearing functional groups such as hydroxylation or nitration retain significant antibacterial effects and are suspected to contribute to the development of antibiotic resistance in bacteria. Due to the homologous series of sulfonamides and their common reactive site, there is much to suggest that similar transformations occur for sulfonamide homologs other than SMX. Given that about a dozen of sulfonamide antibiotics are frequently occurring in surface waters, the screening for all transformation products using target methods would require to purchase or synthesize all potential TPs and would evidently cause unreasonably high costs and is therefore hardly realistic. For this reason and since accurate mass LC/MS/MS is not widespread in control labs, the aim of the present study was to develop a generic scheme to predict the 28 most likely TPs of a sulfonamide by identification of the major transformation reactions and pathways. The mass shifts of each transformation were used to calculate the precise ions for a targeted suspect screening with unit mass LC-MS/MS. The additional use of accurate mass LC/TOF analysis allowed for the tentative identification of TP precursors by their molecular weight and isotopic pattern. Based on the structural elucidation of the parent sulfonamide ion spectrum, mass shifts additionally allowed to predict five characteristic product ions for each derived TP. The prediction scheme was validated using polycyclic aromatic hydrocarbons (PFAAs), activic pharmaceutical ingredients and polar pesticides. POP analysis was carried out with gas chromatography and high resolution mass spectrometry. Compounds with higher polarity were analyzed with micro-scale ultra-performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer. In general, analyses seemed to occur in comparable concentrations in different water depth. Individual pharmaceutical residues and polar pesticides were detected at concentration levels between 25 and 4500 pg L⁻¹. PFAs were found in a concentration range between 0.01 and 1300 pg L⁻¹. PCBs and PAHs were mostly below the detection limit (0-400 pg L⁻¹. OCPs could be detected at trace levels between about 0 and 70 pg L⁻¹, individual PCB concentrations were in the low pg L⁻¹-scale and thus mostly in the range of blank values.

**TU195**

**Identification of diphenyl-bis(trimethylisilyl)silane as an environmental contaminant**

A.H. Kreegergaard, M.S. McLachlan, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES.

In an earlier model-based screening study aimed at identifying organosilicon chemicals present in the environment, three previously unknown environmental contaminants were identified. A phenyl substituted siloxane,
phenyl-tris-(trimethylsiloxy)silane, was the most abundant of the three, with concentrations in the range of 50 pg m⁻³ in air and 0.5 ng g⁻¹ dw. in sediment from the Stockholm archipelago. Other phenyl substituted organosilicones have been prioritized as possible persistent and bioaccumulative chemicals based on their properties. To further explore this group of chemicals, several contaminated sediment and sewage sludge samples were screened. Full scan spectra of a number of semi-volatile phenylsilicones were obtained from purchased standards and from public MS libraries. Common fragmentation patterns were identified and SIM descriptors for phenylated linear and cyclic silicones were selected. Sediment and sewage sludge were solvent extracted with dichloromethane/acetone and cleaned up with a purge & trap method on an ENV+ SPE-cartridge. The samples were analysed using GC/MS. From these analyses dimethyl-bis(trimethylsiloxy)silane was identified and positively confirmed in sediment from Stockholm harbor and in sewage sludge from two sewage treatment plants in Stockholm.

TU196 Risk evaluation of Fipronil in freshwaters from State of São Paulo, Brazil

C.C. Raimundo, UNICAMP / Institute of Chemistry; A.J. Nogueira, University of Aveiro / Departamento de Biologia CESAM; A.F. Albuquerque, University of Campinas; G.d. Umbuzeiro, FACULTY OF TECHNOLOGY - UNICAMP / LEAL.

Fipronil is a broad-spectrum phenylpyrazole insecticide. It is used as active ingredient in pesticides formulation for agriculture applications and as parasiticide for pets. So fipronil is a contaminant that can be found in surface waters from both rural and urban areas. This study presents an investigation into the potential for ingredients used in PCPs and DCPs to enter the environment through their typical use and entry to sewerage and subsequent treatment. Three prioritised chemicals, predicted levels of maximum exposure to these same chemicals through their intended use. However, for ten of the thirty-three prioritised chemicals, predicted levels of maximum exposure through drinking water and bathing were close to or greater than would be anticipated through their intended use. These ten prioritised chemicals included, chelating agents (phosphonates, EDTA), boric acid, surfactants (linear alkylbenzene sulphonate and cocamidopropyl betaine), 2-(2-Butoxyethoxy)ethanol (DEGBE) and betaoxirane. This provides a focus for further work that may be required to assess their potential for PCPs and DCPs to occur in drinking water in the UK. The research was funded by Defra and managed by the Drinking Water Inspectorate (DWI). The views expressed here are those of the authors and not necessarily those of the Defra or the DWI.

TU198 Orthogonal, parallel Fractionation of Androgens and Antiandrogens by Liquid Chromatography

M. Muschiet, UFZ - Helmholtz Centre for Environmental Research / EffectDirected Analysis; M. Krauss, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; A. Bahmann, Helmholtz-Zentrum für Umweltforschung UFZ / Effect Directed Analysis; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis.

Effect Directed Analysis (EDA) is a powerful approach for the identification of potentially harmful compounds in environmental compartments like sediments or water. The success of EDA is essentially influenced by the fractionation procedure. This is classically performed in sequence of LC fractionation, biotesting and selection of active fractions for the next LC fractionation step until the complexity is lowered to a small number of compounds, for which structure elucidation is carried out. We propose a parallel fractionation of a sample on orthogonal columns as a time-efficient alternative. Subsequently chemical and toxicological analysis is performed on fractions from the different separation systems. Here candidate peaks shared by bioactive fractions are selected and subjected to structure identification. To test the applicability of this approach, an EDA on androgens and antiandrogens was chosen as a case study. The selection of orthogonal columns was based on a set of 52 known or suspected androgens and antiandrogens. The retention times of these compounds were determined on 16 different stationary phases owning widely differing chemistries. Those three columns allowing for the best orthogonal separation were selected for parallel fractionation of a surface water sample.

TU199 The NORMAN interlaboratory study on biotesting of spiked water extracts

C. Di Paol, RWTH Aachen University / Ecosystem Analysis ESA; S.H. Keiter, Oerbro University / MTM Research centre; S. Ait-Assia, INERIS / Ecotoxicology Institute of Technology; F. Baltes, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis; M. Breitling, Inst for tillamped miljövetenskap / Department of Applied Environmental Science ITM; S. Buchinger, Federal Institute of Hydrology / Department G Biochemistry Ecotoxicology; C. Chalon, Issp, B. COUSIN, INRA; V. Dulio, INERIS; B. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ - Cell Toxicology; T. Hamers, VU University Amsterdam. Then we investigated the occurrence of fipronil in sub-nanogram per liter levels, in 42 samples of different water bodies located in both agricultural and urban areas from São Paulo State. Then we calculated a quality water criteria for the protection of the aquatic life using species sensitivity distribution (SSD). The calculated criteria were used to verify the risk of fipronil to aquatic life. Fipronil extraction was performed using 1-Liter samples using solid phase extraction. Quantification was performed by liquid chromatography coupled to mass spectrometry (LC-MS/MS). The Limit of Quantification for the analytical method (LOQ) was 0.8 ng L⁻¹, considering the concentration factor of 1,000 times. Fourty two samples were collected during February to October 2014 from 14 different rivers and reservoirs from São Paulo State. A preliminary value for the PNEC was derived from the dataset available at the ECOTOX database. The HC5 (hazard concentration at 5 %) was calculated with the SSD Generator from EPA. The PNEC value was calculated according to the recommendations of the European Chemical Bureau and assessment factor of 2 was used Fipronil was present in 100 % of the samples in concentrations from 1.2 and 21.7 ng L⁻¹. A value of 192 (84-437) ng L⁻¹ as HC5 (50 % CI) resulted, after application of the assessment factor of 2 in a PNEC (50 % CI) = 96 (42-219) ng L⁻¹. The goodness of fit for the estimation of the HC5 was 0.973 (n=46). A risk Quotient (RQ) was calculated for each of the sampling sites dividing the measured environmental concentration (MEC) by the PNEC value. Although fipronil was present in 100 % of the freshwaters collected in São Paulo State, risk quotient was always under 1 but in some areas the MEC of fipronil rise concern for the coming future. Monitoring studies are needed to verify if the occurrence of fipronil, and its transformation products, are likely to raise environmental concern, not only in water but also in sediments and biota in the São Paulo State.

TU197 Risks to drinking water from Personal Care Products and Domestic Cleaning Products

V. Benson, WRC plc / National Centre for Environmental Toxicology; T. Dee, A. Ewence, R. Gee, WRC plc; T. Hall, W. Mark, L. Rockett, WRC plc; C. Watts, Chris Watts Associates Ltd.

Personal care products (PCPs) and domestic cleaning products (DCPs) are a diverse range of products that contain vast numbers of different chemicals. These chemicals may have the potential to reach drinking water supplies via release into the environment through typical use and entry to sewerage and subsequent treatment. The potential threat of exposure to these chemicals to drinking water and bathing was assessed. To test the applicability of this approach, an EDA on androgens and antiandrogens was chosen as a case study. The selection of orthogonal columns was based on a set of 52 known or suspected androgens and antiandrogens. The retention times of these compounds were determined on 16 different stationary phases owning widely differing chemistries. Those three columns allowing for the best orthogonal separation were selected for parallel fractionation of a surface water sample.

TU198 Orthogonal, parallel Fractionation of Androgens and Antiandrogens by Liquid Chromatography

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Effect Directed Analysis (EDA) is a powerful approach for the identification of potentially harmful compounds in environmental compartments like sediments or water. The success of EDA is essentially influenced by the fractionation procedure. This is classically performed in sequence of LC fractionation, biotesting and selection of active fractions for the next LC fractionation step until the complexity is lowered to a small number of compounds, for which structure elucidation is carried out. We propose a parallel fractionation of a sample on orthogonal columns as a time-efficient alternative. Subsequently chemical and toxicological analysis is performed on fractions from the different separation systems. Here candidate peaks shared by bioactive fractions are selected and subjected to structure identification. To test the applicability of this approach, an EDA on androgens and antiandrogens was chosen as a case study. The selection of orthogonal columns was based on a set of 52 known or suspected androgens and antiandrogens. The retention times of these compounds were determined on 16 different stationary phases owning widely differing chemistries. Those three columns allowing for the best orthogonal separation were selected for parallel fractionation of a surface water sample.
Acute aquatic toxicity screening of cellulosic products contained glyoxal, using multi-strain bacteria, daphnia, and zebrafish embryos

C. Park, Korea Institute of Science and Technology Europe / Convergence Environment Team Environment Bio Group; M. Song, Samsung Fine Chemicals / Green Materials Development Team; N. Choi, Samsung Fine Chemicals / Green Material Development Team; S. Kim, KIST Europe / Chemical Management Lab

It is well known that cellulose ethers are water-soluble polymers derived from cellulose which is consist of natural organic compounds. These polymers have been specifically modified in a wide range of applications, from construction products, ceramics, paints, personal care products, and pharmaceuticals. From June 2015, the information about the safety of cellulosic products contained an additives such as a cross-linking agent (i.e., glyoxal) on the human- and environment health will need to response in EU CLP (classification, labelling and packaging) legislation. To screen the aquatic-toxic effects of cellulosic products involved glyoxal with different concentrations, the acute aquatic toxicity test using multi-strain environmental bacteria (microbial array for toxicity risk assessment, MARA), Daphnia magna (OECD test guideline 202) and zebrafish embryos (OECD test guideline 203) was conducted in this study. The acute aquatic toxicity using 3 different aquatic organisms (multi-strain bacteria, Daphnia magna, and zebrafish embryos) were limit and not significant. To better evaluate the aquatic-toxic effects of cellulosic products contained cross-linking agents, the chronic toxicity test will be performed and the further studies regarding the toxic effects of cellulosic products by physical properties (e.g., solubility, viscosity, pH change, etc.) is also required.

Are phytoestrogens and mycoestrogens relevant emerging pollutants?

B. Jarosova, Masaryk University / Faculty of Science RECETOX; J. Javurek, Masaryk University / Faculty of Science RECETOX; K. Hilscherova, Masaryk University, Faculty of Science, RECETOX / RECETOX Research Centre for Toxic Compounds in the Environment

Phytoestrogens and mycoestrogens enter surface waters from both natural and anthropogenic sources and could potentially contribute to estrogenic effects in aquatic biota. In this study, available data on their estrogenic potencies and detected concentrations in surface waters were used in the risk assessment concerning their potential adverse effects. The study also indicates the potential extent of use of different assays to detect the phyto/mycoestrogens at their effective concentrations. The study was supported by the Czech National Science Foundation grant No. 5P05/12/0553 and EU FP7 project SOLUTIONS (grant No. 603437).

Use of fish farms to assess river contamination: combining biomarker responses, active biomonitoring, and chemical analysis.

J. Navas, INIA / Madrid; M. Fernandez Cruz, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) / Departamento de Medio Ambiente Lab DETEC; A. Quesada-Garcia, INIA; M. Villarroel, Universidad Politecnica de Madrid; M. Gomez, University of Queensland; M. Hernandez, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA); F. Torrent, Technical Superior School of Forest Engineers Polytechnic University of Madrid; A. Valdés, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) / Departamento de Medio Ambiente Lab DETEC

Aquatic organisms are often exposed to mixtures of low levels of pollutants whose presence and effects can pass easily unnoticed if only traditional monitoring strategies are employed. In this regard, the combination of different techniques is essential to detect the possible effects of trace levels of contaminants on fish. In this study biomarkers response, active biomonitoring (ABM) and chemical analysis were used. In two freshwater fish species, the presence of micropollutants (PAHs and CyP450 and related enzyme activities (7-ethoxyresorufin-O-deethylase, EROD, and benzoyloxy-4-[trifluoromethyl]-coumarin-O-debenzyloxylase, BFCOD, respectively) were used to test for exposure to a variety of contaminants. Biomarker methodologies applied on fish farms has recently emerged as a realistic method to assess any exposure of fish to chemical compounds that could potentially disturb their modifications of multiple metabolic and homeostasis. In two rainbow trout (Oncorhyncus mykiss) fish farms that are routinely sampled we observed a strong and increase in these activities at two different moments. In one case this increment was accompanied by antiestrogenicity phenomena. In order to shed light on the causes of this induction, in an ABM approach we transferred some fish to a fish farm with controlled conditions and examined them. EROD activity showed a decrease of 80% from the original values after only 7 days in the control farm, while BFCOD activity was also reduced after 15 days. Although not significant, a decrease in cyP450 and cyP3A mRNA levels was also observed. To determine the presence of pollutants, water and sediment samples from the river feeding the fish farms were analyzed by two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–TOF MS). Trace levels of chlorinated and fluorinated hydrocarbons were identified in the samples collected in the fish farms, indicating that the observed effects in fish should be caused by the (combination of) accompanying substances. The methodology here described has proven useful in detecting trace levels of contaminants in water courses and elucidating their influence on biota.

Estimating baseline toxicity and in vitro and in vitro bio-pollution of mixture of non-polar analytes using GC×GC

D. Nabi, Masdar Institute / iWater; J. Gros, LMCE; J.S. Arey, EPFL / LMCE

Existing estimation methods are generally not successful for the estimation of aquatic baseline toxicity and bio-pollution properties of thousands of potentially bioaccumulative contaminants found in complex mixture of nonpolar analytes. Ideally, these estimation approaches should be targeted at compounds actually measured in environmental samples. In the present study, we showed that aquatic baseline toxicity and bio-pollution properties can be mapped onto the separation space offered by comprehensive two-dimensional gas chromatography (GC×GC). We used GC×GC chromatogram retention information to estimate baseline toxicity and bio-pollution properties for a diverse set of non-polar halogenated and hydrocarbon compounds that undergo negligible or limited hydrogen bonding interactions. When compared to the experimental data our model exhibited root mean square error (rmse) of 0.32 log unit for 96-hour median lethal concentration (-log LC50) endpoint for fathead minnow. We developed the toxicity testing of the modeling for complex mixtures of thousands of potentially bioaccumulative contaminants found in complex mixture of nonpolar analytes. Ideally, these estimation approaches should be targeted at compounds measured in environmental samples. In the present study, we showed that aquatic baseline toxicity and bio-pollution properties can be mapped onto the separation space offered by comprehensive two-dimensional gas chromatography (GC×GC). We used GC×GC chromatogram retention information to estimate baseline toxicity and bio-pollution properties for a diverse set of non-polar halogenated and hydrocarbon compounds that undergo negligible or limited hydrogen bonding interactions. When compared to the experimental data our model exhibited root mean square error (rmse) of 0.32 log unit for 96-hour median lethal concentration (-log LC50) endpoint for fathead minnow. We developed the toxicity testing of the modeling for complex mixtures of thousands of potentially bioaccumulative contaminants found in complex mixture of nonpolar analytes. Ideally, these estimation approaches should be targeted at compounds measured in environmental samples. In the present study, we showed that aquatic baseline toxicity and bio-pollution properties can be mapped onto the separation space offered by comprehensive two-dimensional gas chromatography (GC×GC). We used GC×GC chromatogram retention information to estimate baseline toxicity and bio-pollution properties for a diverse set of non-polar halogenated and hydrocarbon compounds that undergo negligible or limited hydrogen bonding interactions. When compared to the experimental data our model exhibited root mean square error (rmse) of 0.32 log unit for 96-hour median lethal concentration (-log LC50) endpoint for fathead minnow. We developed the toxicity testing of the modeling for complex mixtures of thousands of potentially bioaccumulative contaminants found in complex mixture of nonpolar analytes. Ideally, these estimation approaches should be targeted at compounds measured in environmental samples. In the present study, we showed that aquatic baseline toxicity and bio-pollution properties can be mapped onto the separation space offered by comprehensive two-dimensional gas chromatography (GC×GC). We used GC×GC chromatogram retention information to estimate baseline toxicity and bio-pollution properties for a diverse set of non-polar halogenated and hydrocarbon compounds that undergo negligible or limited hydrogen bonding interactions. When compared to the experimental data our model exhibited root mean square error (rmse) of 0.32 log unit for 96-hour median lethal concentration (-log LC50) endpoint for fathead minnow. We developed the toxicity testing of the modeling for complex mixtures of thousands of potentially bioaccumulative contaminants found in complex mixture of nonpolar analytes. Ideally, these estimation approaches should be targeted at compounds measured in environmental samples.

Risk assessment associated with the presence of emerging organic micropollutants originated from municipal wastewater in the aquatic environment: the case of Greek rivers

V.S. Thomaidi, University of the Aegean / Department of Environment; A.A. Maziotti, Aegean University / Environment; A.S. Stasinakis, University of the Aegean / Department of Environment; N.S. Thomaidis, University of Athens / Department of Chemistry

The purpose of this study was to estimate the environmental risk associated with the presence of emerging organic contaminants, released from treated municipal wastewater, in rivers. For this reason, 25 Greek rivers were chosen as a case study. After literature review, the concentration levels of emerging contaminants that had been detected in Greek rivers were extracted. The extracted data were used to select the most degradable and the experimental acute toxicity data of these compounds (EC50 or LC50 values) for 3 different aquatic organisms (fish, daphnia magna and algae) was collected. In cases that no experimental toxicity data was available, ECOSAR model (U.S.EPA) was used. Taking into account the Maximum Measured Environmental Concentration (MEC), the Predicted No Effect Concentration (PNEC) and the Dilution Factor (DF) the Risk Quotients in rivers (RQs) were calculated. The possible hazard due to the mixture of target micropollutants was estimated as well, using baseline toxicity data from ECOSAR program. According to the results, monitoring data was available for 207 emerging contaminants, belonging to 8 different groups: pharmaceuticals, illicit drugs, phenolic endocrine disruptors (EDCs), perfluorinated compounds,
benzotriazoles, benzothiazoles, artificial sweeteners and siloxanes. The rivers with DF equal to 2 and 3 presented the highest possibility for ecological threat due to the presence of 22 and 19 emerging contaminants, respectively. Amongst target compounds, triclosan presented RQ > 1 for all studied rivers (DF up to 2388), indicating a possible ecological risk for algae regardless wastewater dilution. Additionally, decamethylcyclopentasilane (in daphnia magna), caffeine (in algae) and nonylphenol (in fish) were identified as high risk contaminants (RQ > 1), while for 22 and 19 emerging contaminants, respectively. The class of predicted pollutants with the highest risk was EDCs, since 4 substances (nonylphenol, nonylphenol diethoxylate, nonylphenol monoethoxylate and triclosan) presented high RQ values even after wastewater dilution. The mixture of the emerging contaminants presents a serious risk in all studied rivers, as the mixture’s RQ had varied between 1 and 17 (99 risk for fish, 123 risk for algae) even in river with the highest DF. EDCs present the greatest contribution to the toxicity of the mixture (96% in fish and daphnia magna and 98% in algae).

TU205 Risk Based Prioritisation of Pharmaceuticals in the environment in Iraq O. Abdullah, York University / Environment; A. Boxall, University of York / Environment Department Numerous studies have shown that residues of pharmaceuticals occur in the natural environment, raising concerns about their impact on non-target organisms or human health. One region where little is known about the exposure and effects of pharmaceuticals is on the environment is Iraq. Due to the high numbers of pharmaceuticals used by the public health sector in Iraq (hospitals and care centres), there is a need for a systematic approach for identifying substances that should be monitored in the environment in Iraq and assessed in terms of environmental risk. In this study a risk-based prioritization approach was used on 99 pharmaceuticals in use in Iraq. Initially, information on the amounts of pharmaceuticals used in Iraq was obtained with the top use medicines found to be paracetamol, amoxicillin and metformin HCl with annual consumption exceeding 20 tons. Use of different monitoring data (water, sediment, sludge and soil) monitoring data of 37 different chemical components, triclosan presented RQ > 1 for all studied rivers (DF up to 2388), indicating a possible ecological risk for algae. Identification of new substances of concern. The scientific and technical objective of the European Water Framework Directive (WFD) is to achieve good chemical status by monitoring priority substances (PS) at Union level which are expected in more than one step, and challenges are likely to be faced, while the potential of a substance can be used to more accurately predict the risk of volatile compounds transferred to humans and environment at the far field. “Detection in the Arctic” is not necessarily an indication of high LRTP of the chemical, but may reflect the usage patterns, emission rates and scenario, and sampling and analytical details that have little to do with the transport processes.

TU208 Calculating ‘exposure potential’ - parsimonious approach of the unit-world concept D. van de Meent, RIVM / DMG; J. Struijs, Laboratory for Ecological Risk Assessment; A. Striffler, J. Baumeister, denkbares; D. Stättler, Umweltbundesamt; N. Aust, Federal Environment Agency (UBA) / Biocidence. Parsimony has proven its invaluable in understanding and predicting environmental fate of chemicals. One of the most prominent examples is the unit-world based mass balance modeling of exposure of man and ecosystems to chemical substances, which provides insight how and why chemicals with different physical and chemical properties behave differently. The concept has recently been rediscovered by the German Federal Environment Agency (UBA) / Biocidence in calculating ‘exposure potentials’ of chemicals in air, water and soil for regulatory purposes. Exposure potential of a substance - the same entity is known and used in Life Cycle Impact Assessment (LCIA) as ‘environmental fate factor’ - is defined as the steady-state mass in the environment (kg), per unit rate of release (kg/d) into it. It is calculated by means of a simple, box model (‘simple/boxtreat’): a minimalized (i.e. air, water, sediment, soil) unit-world box model. When the (new) boxtreat is now used by UBA risk assessors to understand and predict how transport and transformation affect exposure levels (ed of new) chemicals in the environment. The simple/boxtreat is used on a routine basis, embedded in UBA’s KnowSEC decision support system. The tool is made available to environmental science students and other users via RIVM’s website (www.rivm.nl). The poster describes and explains the simple/boxtreat model and illustrates this with a regulatory case study.

TU209 Overview of the Prioritisation Process under the Water Framework Directive L. Ceriani, European Commission DG Joint Research Centre / Institute for Environment and Sustainability IES; R. Negro Carvalho, Institute for Environment and Sustainability / Institute for Environment and Sustainability IES; A. Ippolito, EFSA - European Food Safety Authority / Institute for Environment and Sustainability IES; D. Moreau, JRC, EC / Institute for Environment and Sustainability; R. Loos, European Commission DG Joint Research Centre / Institute for Environment and Sustainability; L. Lettieri, European Commission - Joint Research Centre / Institute for Environment and Sustainability The objective of the European Water Framework Directive (WFD) is to achieve good chemical status by monitoring priority substances (PS) at Union level which should not exceed Environmental Quality Standards (EQS). Annex I of the Directive 2013/39/EU lists forty-five PS and Priority Hazardous Substances (PHS), which have been selected amongst those which present a significant risk to or via the aquatic environment. The revision of the PS list has been launched recently with the aim of identifying new substances of concern. The scientific and technical methodology for the current review has been developed by the JRC in consultation with DG ENV and the sub-group of experts of the Working Group Chemical. In fact, a defined prioritisation scheme has been created for both the modelling and monitoring-based exercises. The two exercises will be run in parallel, and will consider more than six-thousands of substances, collected from several publications and databases. More specifically, substances having monitoring data from at least four Member States will be considered for the modelling-based exercise, based on the Spatial, Temporal, and Extent of exceedances frequencies approach (STE approach), developed by the JRC. Whilst all other substances will undergo through the modelling-based scheme, which encompasses firstly a screening phase, based on defined hazard and exposure criteria, and secondly a modelling-based risk assessment (RA). In both the monitoring and modelling-based exercises, the RA will use three separately for (moat) water, sediment, sludge and soil, the typical scenarios of secondary poisoning, and drinking water. Connections between the two exercises are expected in more than one step, and challenges are likely to be faced, while handling different classes of substances, like pharmaceuticals, mixtures, and inorganic compounds.

TU210 A novel device for on-site large volume solid phase extraction for chemical and effect-based monitoring of water resources T. Schulze, A. Klöö, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; A. Bahmann, Helmholtz-Zentrum für Umweltforschung UFZ /
Directed Analysis; M. Krauss, Helmholz Centre for Environmental Research UFZ / Effect Directed Analysis; G. Wall, MAXX Mess und Probenahmetechnik GmbH; W. Brack, Helmholz Centre for Environmental Research UFZ / Effect Directed Analysis

For the implementation of multi- and non-target as well as effect-based analysis in monitoring strategies an innovative and automated solid phase extraction (SPE) based active sampling device was developed. While no or only low enrichment is required for most of the chemicals, the screening of modern analytical equipment, enrichment of large amounts of water is necessary to conduct in vitro and in vitro bioassays or even effect-directed analysis. The newly developed mobile device permits the on-site collection of large water volumes up to 1000 L. This approach avoids the storage and transport of large volumes as well as form the laboratory. Therefore, time-, discharge- or flow-proportional or event based sub-sampling of the water body over days or weeks is made possible in order to yield representative samples. In this paper we present the results of the evaluation of the chemical performance of the large volume solid phase extraction device for 50 L (LVSP50) device in laboratory and field scale [1, 2]. A novel device for on-site large volume solid phase extraction was developed and tested in laboratory and field scale. The laboratory test showed very good recoveries for a broad range of compounds. The field test resulted in methodical detection limits in the lower ng/L range. The device will help to improve monitoring of organic pollutants in water resources in the future. [1] Schulze, T.; Krauss, M.; Bahlmann, A.; Hug, C.; Walz, K.-H.; Brack, W. 2014. Onsite large volume solid phase extraction – how to get 1000 liters of water into the laboratory? SETAC Europe 24th Annual Meeting, 11-15 May 2014 (in press) [2] Schulze, T.; Klödt, A.; Lochen, T.; Hug, C.; Bahlmann, A.; Althheim, J.; Wannenmacher, T.; Haid, U.; Walz, K.-H.; Krauss, M.; Brack, W. in prep. A novel device for on-site large volume solid phase extraction for multi-target, non-target and effect-based screening and monitoring of water resources. Anal Bioanal Chem. In preparation...

TU211
High throughput toxicity screening of the priority substances in water quality assessment
E. Simon, Centre Ecotoxic; M. Naderman, Biodetection Systems BV; B. van der Burg; A. Brouwer, Biodetection Systems BV; P. Behnisch, Biosituation; B. van Vugt-Lussenburg, Biodetection Systems BV;

The list of routinely monitored chemicals in European water bodies is compiled based on certain selection parameters (e.g. production volume, occurrence, pose significant risk to or via the aquatic environment), however, their toxic potency (involving broad range of toxic pathways) has not been investigated extensively before. In addition, water samples can contain many toxic compounds, their metabolites and degradation products which are not included in current regulations. Bioassays can be used to assess the effect of the entire mixture of compounds, thereby reducing uncertainty in safety evaluation. In order to harmonize and combine current monitoring practices (chemical analytics) with innovative effect-based bioanalytical tools with our study we aimed at the: i) Compilation of the extensive toxicity profile of the routinely monitored substances (priority and non-priority) and substances that are under review for being listed as pollutants of emerging concern – ii) Establishment of overlap and complementarity of the chemical- and biological analytical data. A total number of approximately 90-100 compounds are currently being screened in the high throughput in vitro reporter gene bioassays (CALUX® panel of 24 assays) covering a wide range of toxic endpoints, such as endocrine disruption, xenobiotic metabolism, genotoxicity, oxidative stress, disturbances of lipid metabolism. Preliminary results with 24 compounds revealed so far the importance of endocrine (particularly the activation of the estrogen receptor, and antagonistic activity on the androgen- and progesterone receptors) and xenobiotic metabolism-related (activation on the aryl hydrocarbon receptor) pathways of the routinely monitored substances. Detailed comparison of these results of the environmentally relevant samples and regulated substances will be presented to identify strength- and weaknesses of both approaches.

TU212
Screening for pharmaceuticals and other emerging pollutants in sediments of major European estuaries

Pharmaceuticals and many other chemicals that became pollutants of emerging concern in the environment are indispensable goods in our society. They are so far not included in systematic monitoring programs and their fate in and effect to the environment is largely unknown. Due to their physico-chemical properties these substances may accumulate in sediments. Thus, sediments serve as a large sink. In this study sediments from 13 river estuaries in Europe are analyzed for the extent of contamination with pharmaceuticals and other emerging pollutants. A wide range of target compounds covering different chemical and effect classes was selected considering predicted persistence and bioaccumulation potential (EpiSuite), physico-chemical properties such as logKw, and pKa, values, production volumes and information on authorization and sales amounts in the EU. Additionally, compounds detected in previous sediment studies were included. A multi-target method has been developed using pressurized liquid extraction and clean-up by adsorption chromatography and liquid-liquid extraction. For the analysis of sediment samples a novel screening approach using liquid chromatography – high resolution mass spectrometry and gas-chromatography-mass spectrometry was employed. These techniques do not only allow for target analysis but also for the detection of the metabolites. For the target pharmaceuticals the method detection limits ranged between 0.2 – 52.6 ng/g. The recovery varied from less than 20% to greater than 100% whereas most compounds showed a recovery greater than 50%. The matrix effect ranged between -78% to 65% with a median suppression of -8%. So far, sediments from the Danube delta (Ukraine and Romania) as well as from the Po River in Italy have been subject to target analysis for pharmaceuticals. In total, eight out of 60 target compounds were detected in the sediment samples including amitryptiline, citalopram, flutamide, miconazole, propanolol, sertraline, terbinafine and tramadol. The study will contribute to the knowledge of the fate of pharmaceuticals and other emerging chemicals in the environment and shed light on the question whether pollution patterns are river basin specific or if an EU-wide contamination is observed.

TU213
Quantitative structure-biodegradation relationship (QSBR) development and thermodynamic analysis to understand the biodegradation rates of chemicals k. scharya, Newcastle University / Environmental Engineering; J. Dolfin, Newcaste University / Civil Engineering; M. Naderman, Biodetection Systems BV; B. van der Sillero, IDAEA; B. van der Burg; A. Brouwer, Biodetection Systems BV; P. Behnisch, Biosituation; B. van Vugt-Lussenburg, Biodetection Systems BV

Directed Analysis; M. Krauss, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis; G. Wall, MAXX Mess und Probenahmetechnik GmbH; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis; G. Wall, MAXX Mess und Probenahmetechnik GmbH; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis; G. Wall, MAXX Mess und Probenahmetechnik GmbH; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis; G. Wall, MAXX Mess und Probenahmetechnik GmbH

The newly developed mobile device permits the on-site large volume solid phase extraction and tested in laboratory and field scale. Therefore, time-, discharge- or flow-proportional event based sub-sampling of the water body over days or weeks is made possible in order to yield representative samples. In this paper we present the results of the evaluation of the chemical performance of the large volume solid phase extraction device for 50 L (LVSP50) device in laboratory and field scale [1, 2]. A novel device for on-site large volume solid phase extraction was developed and tested in laboratory and field scale. The laboratory test showed very good recoveries for a broad range of compounds. The field test resulted in methodical detection limits in the lower ng/L range. The device will help to improve monitoring of organic pollutants in water resources in the future. [1] Schulze T, Krauss M, Bahlmann A, Hug C, Walz K-H, Brack W. 2014. Onsite large volume solid phase extraction – how to get 1000 liters of water into the laboratory? SETAC Europe 24th Annual Meeting, 11-15 May 2014 (in press) [2] Schulze T, Klödt A, Lochen T, Hug C, Bahlmann A, Althheim J, Wannenmacher T, Haid U, Walz K-H, Krauss M, Brack W. in prep. A novel device for on-site large volume solid phase extraction for multi-target, non-target and effect-based screening and monitoring of water resources. Anal Bioanal Chem. In preparation...

TU214
SPE-LC-(APPI)-MS/MS analysis of selected anthropogenic inofchemicals in Mediterranean rivers
D. Garcia-Sillero, IDAEA-CSIC / Analytical Environmental Chemistry; D. Mollino-Delgado, Environmental Chemistry; S. Diaz-Cruz, IDAEA-CSIC / Environmental Chemistry; G. Wall, MAXX Mess und Probenahmetechnik GmbH; W. Mrozik, Newcastle University / School of Civil Engineering; Geoscience; D. Werner, Newcastle University / Civil Eng and Geosciences; R. Davenport, Newcastle University / School of Civil Engineering and Geosciences

QSBR, a relatively recent form of Quantitative Structure Activity Relationship (QSAR) used to predict the rate and/or transformation product of microbial degradation. The majority of QSBRs are developed for congenic chemicals, and mostly incorporate information of their physicochemical properties, but neglect information about the microbial communities participating in their degradation. The broad aim of this study is to develop a new QSBR for a diverse set of aromatic pollutants that have some structural similarity using physicochemical properties of the chemicals and the information of the specific degrading communities. Twenty five aromatic chemicals were selected (20 training set and 5 validation set). First order biodegradation rate estimated using Ultimate Biodegradation rating and Ultimate Biodegradation rating in time from Biowin3 model were used as a response variable. Nine molecular descriptors independent of each other were initially selected as explanatory variables. Multiple Regression Analysis (MRA) was performed using MINITAB 17 Statistical Software, where best subsets regression approach was used to identify the best predictors for the model. In addition, thermodynamic analysis of the biodegradation pathways of individual chemicals was performed using the group contribution method, where the Gibbs free energy of individual reaction in the degradation pathway until aromatic ring was calculated. The QSBR model [Adjusted R² = 0.907 and R² (predicted) = 0.81] developed using MRA incorporated five descriptors (sima, molar refactivity, 1st and 2nd order valence connectivity indices and van der waal Volume). Most notably, total fragment coefficient which is one of the variable in the Biowin3 model; if included in this model, the R² (predicted) increases to 0.914. The thermodynamic analysis of biodegradation pathways of chemicals revealed the role of specific functional groups as well as cleavage of aromatic ring have large negative Gibbs free energy change compared to other reactions in the pathways. Information on the energy yield and the enzyme catalyzing the biodegradation reaction will be instrumental in predicting the activity of degrading microorganisms, which might ultimately be used in QSBR together with those descriptors above. Therefore, future studies will focus in improving the robustness of the QSBR model by incorporating the quantitative information of degrading microbial communities in the model.

Towards a reliable environmental impact assessment of personal care products (P)

Keywords: Inofchemicals, PCPs, chromatography, insect repellents The manipulation of the odor perception of organisms by anthropogenic substances which may result in ecologically relevant behavioural disorder is known as inofchemical effect. It plays an important role in life history, habitat finding, food recognition and survival of a wide range of animals. However, synthetic chemicals potentially interfering such functions we find insect repellents. The widespread application of these compounds to human skin and the subsequent wash-off has been lead to detect concentrations of up to several μg/L in surface waters. In these fresh waters, insect...
repelets might affect aquatic non-target organisms disturbing their behaviour and distribution pattern (communication among individuals, etc.) These personal care products (PCPs) have been typically analysed by GC-MS. The aim of this work was to develop and validate a new method for the analysis of 6 synthetic insect repellent residues in water. The method is based on solid phase extraction, liquid chromatography/tandem mass spectrometry (SPE-LC-MS/MS) using chemically modified (APPI) as ionization mode. The insect repellent residues that have been analysed so far are permethrin compounds such as DEET and Bayrepel (Picaridin) extensively used for decades. In the present study we analysed a new group of repellents, that appear to be good substitutes for DEET. The performance of the method along with an example of their applicability will be presented. References: Kalayci et al., World J Microbiol Biotechnol (2014) 30:407–411; Nendza et al., Environmental Sciences Europe (2015) 25:21. Matamoros et al., Anal Bioanal Chem (2009) 393:847–860. Rodil et al., J Chromatogr. A. 1178 (2008) 9–16. Acknowledgements This work was funded by the EU Project GLOBAQUA (GA 603629) and by the Generalitat de Catalunya (Consolidated Research Group “2014 SGR 418 - Water and Soil Quality Unit”).

TU215 Identification and assessment of photo transformation products of the UV filters Octocrylene, Benzophenone-3 and Ethylhexyl methoxyacaminate present in grey water intended for water reuse
F. Jentzsch, University Lüneberg / INUC; O. Olsson, Leuphana University Lüneburg; K. Kuenmeier, Leuphana University Lüneburg / Institute of Sustainable and Eure and toxicochemical assessment of the formed transformation products. Especially in arid areas decentralized grey water reuse is an important measure to save water. Grey water has a low load of pollutants and therefore has a high potential for reuse such as for irrigation, toilet flushing or laundry. Classical biological treatment of grey water is not able to eliminate micro pollutants such as UV filters substances that originate from personal care products (PCP) (e.g. Eriksson et al. 2005). The discharge of UV filters to grey water prevents their hormonal activities [Kanz and Fent (2006)] and potential of bioaccumulation [Balmer et al. 2005]. As long as full mineralization is not achieved, the suggested photo-oxidation process can result in the formation of transformation products (TP) that may continue to pose environmental or health concerns. For this reason the UV-C treatment as potential post-treatment method for UV filters in grey water was investigated. The primary elimination of three selected UV filters – Octocrylene, Benzophenone-3 and Ethylhexyl methoxyacaminate and identification and evaluation of their photo-TPs was done. Photoysis studies were carried out in a photo reactor by exposing spiked solutions to light of a medium pressure mercury vapor lamp. The primary elimination of the UV filters was analyzed by HPLC-UV/VIS. Moreover, the formation of TP s was followed by HPLC-UV/VIS while the structure elucidation of the generated TPs was ensued via LC-MS/MS analysis. The biodegradation, sorption and toxicity were assessed for the proposed photo-TPs using quantitative structure activity relationships (QSARs). Based on these results, the applicability of photo-oxidation of the selected UV filters was evaluated by investigation of the degree of mineralization, biodegradability, stability and sorption. The new derived information on the degradation efficiency of the selected PCPs as well as the risk assessment of the formed transformation products extends the knowledge on sustainable grey water treatment options. References: Balmer, M. E.; Buser, H.-R.; Müller, D. M.; Poiger, T. (2005) Environ. Sci. Technol. 39 (4): 953–962. Eriksson, E.; Donner, E.; Ledin, A. (2010) Water Science & Technology 62 (12): 2889-2898. Kunz, P. Y.; Fent, K. (2006) Aquat. Toxicol. 79: 305-324.

TU216 Method Comparison for the Analysis of Cyclic Volatile Methyl Siloxanes in Surface Water and Waste Water Treatment Plant Effluent
S.M. Kroser, Dow Corning Corporation / Health and Environmental Sciences; Y. Horii, Center for Environmental Science in Saitama; K. Kobayashi, N. Suganuma, Silicone Industry Association of Japan; J.A. Durham, Dow Corning Corp. / Health and Environmental Sciences; T.H. Schramke, Dow Corning Corporation / Health and Environmental Sciences
Cyclic volatile methyl siloxanes (cVMS) are common ingredients in many consumer products, including personal care products such as hair conditioners and deodorants. They are used as UV filters in cosmetics and sunscreens. They are hydrophobic, tend to sorb to sediments and are not biodegradable. The presence of UV filters in grey water intended for water reuse is a major concern in the urban environments of Barcelona city and its surroundings in terms of occurrence and ecotoxicological potential risk posed by the target PCPs in the sampling sites. Eight UV-Fs were selected on the basis of their high production rates, extensive use and potential ecotoxicological effects. Surface water and wastewater were analysed to assess the levels of the target UV-Fs, as well as to estimate the removal efficiency of greywater. Population growth and uncontrolled occupation of irregular areas are a major problem in this region, leading to the degradation of aquatic ecosystems. Studies using caffeine concentration as a tracer for antrhopic activities were conducted in the region [1] indicating intense anthropogenic influence, attributed to the presence of domestic sewage. Recently, researches on emerging organic micropollutants, such as UV-Fs, stimulated their scientific interest. These contaminants are used in personal care formulations to absorb solar radiation to protect against its harmful effects, also used in many industrial processes and stability of products. Several studies revealed their potential toxicity and endocrine disrupting capacity, showing adverse effects on fecundity and reproduction in organisms [2]. Many UV filters are high lipophilic and low degradable, tending to accumulate in sediments [3], sewage sludge [4] and aquatic organisms [5]. In the present study the occurrence of UV filters was investigated for the first time in sediment samples from Iguaçu basin. Seventeen sediment samples were analyzed to determine 20 UV filters and derivatives. Samples were collected in 3 urban rivers, Belém, Palmital and Atuba River, in two sampling campaigns in 2014 in Metropolitan Region of Curitiba. The contaminants were extracted using a method based on modification of previous high performance liquid chromatography tandem mass spectrometry (HPLC-QqQ-LTQ-MS/MS). Acknowledgements: Miquelina thanks CAPES foundation, Ministry of Education of Brazil, for the scholarship (Process number 0137-14-6). We also thank the Generalitat de Catalunya (Consolidated Research Group “2014 SGR 418 - Water and Soil Quality Unit”).

TU217 Occurrence And Environmental Impact Of Organic UV Filters In Urban Aquatic Ecosystems
D. Molina Delgado, Environmental Chemistry; J. Távora, S. Díaz-Cruz, IDAEA-CSIC / Environmental Chemistry; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research
Organic UV Filters (UV-Fs) constitute a diverse group of chemicals used as a protection against the harmful effects of the UV sunlight. We can find them in many daily use products such as personal care products or as additives in polymeric materials that need protection against sunlight. The quantities of these substances that enter the environment are particularly important because, in opposition to many other emerging contaminants, PCPs have an uncontrolled flow to the receiving systems through activities like swimming and bathing, spraying and excretion. As a consequence of their continuous release into the environment and their poor degradability and limited elimination in WWTWs [2] they are considered persistent contaminants [1]. Therefore, the study and understanding of these substances in the environment is of major importance because of their potential to bioaccumulate and consequent toxicity, with potential negative impact especially on biota, or even on humans [3, 4]. The aim of this work is to analyse the impact of UV-Fs in the urban environments of Barcelona city and its surroundings in terms of occurrence and ecotoxicological potential risk posed by the target PCPs in the sampling sites. Eight UV-Fs were selected on the basis of their high production rates, extensive use and potential ecotoxicological effects. Surface water and wastewater were analysed to assess the levels of the target UV-Fs, as well as to estimate the removal efficiency of greywater. Population growth and uncontrolled occupation of irregular areas are a major problem in this region, leading to the degradation of aquatic ecosystems. Studies using caffeine concentration as a tracer for antrhopic activities were conducted in the region [1] indicating intense anthropogenic influence, attributed to the presence of domestic sewage. Recently, researches on emerging organic micropollutants, such as UV-Fs, stimulated their scientific interest. These contaminants are used in personal care formulations to absorb solar radiation to protect against its harmful effects, also used in many industrial processes and stability of products. Several studies revealed their potential toxicity and endocrine disrupting capacity, showing adverse effects on fecundity and reproduction in organisms [2]. Many UV filters are high lipophilic and low degradable, tending to accumulate in sediments [3], sewage sludge [4] and aquatic organisms [5]. In the present study the occurrence of UV filters was investigated for the first time in sediment samples from Iguaçu basin. Seventeen sediment samples were analyzed to determine 20 UV filters and derivatives. Samples were collected in 3 urban rivers, Belém, Palmital and Atuba River, in two sampling campaigns in 2014 in Metropolitan Region of Curitiba. The contaminants were extracted using a method based on modification of previous high performance liquid chromatography tandem mass spectrometry (HPLC-QqQ-LTQ-MS/MS). Acknowledgements: Miquelina thanks CAPES foundation, Ministry of Education of Brazil, for the scholarship (Process number 0137-14-6). We also thank the Generalitat de Catalunya (Consolidated Research Group “2014 SGR 418 - Water and Soil Quality Unit”).

TU218 Sediments as a sink for UV Filters: The case study of Upper Iguaçu basin, Curitiba (Brazil)

TU219 Size and amount of microplastics in toothpaste
A.J. Verschoor, RIVM / Centre for Safety of Substances and Products; J. Hermanssen, RIVM / National Institute for Public Health and the Environment /
Micropolastics may be used in cosmetics; for example in rinse-off products such as soap, shower and bath products, facial cleaners and toothpaste. The main reason to add micropolastics in these products is their abrasive or polishing effect, and sometimes also slimming effect. The micropolastics in rinse-off products enter household wastewater. Some studies in The Netherlands have shown that these particles may pass the sewage treatment plants and enter the surface water, where they are consumed by aquatic organisms. The ecological risk assessment of micropolastics in the aquatic environment has to deal with many uncertainties in relation to fate and effects. However, the large-scale presence and persistence of (micro)plastics are undebated and from a precautionary point of view, actions to reduce their environmental impact are required. Micropolastics are considered as a potential criterion for allowance and enforcement. However, only limited information is publicly available about the actual particle sizes and amounts of micropolastics in rinse-off products. Company information and public literature was collected regarding the size of micropolastics in facial scrubs and bath gellers. In addition laboratory tests were performed on three toothpastes from different brands to investigate the size and amount of polyethylene speckles. The poster describes the methods used for analyses of particles size distribution and chemical confirmation of polymer identity, and will present an overview of the collected data.

TU220
Photochemical transformation of climbazole under artificial UV light and sunlight: Identification and evolution profile of photo degradation by-products
G. Alvarez-Rivera, Department of Analytical Chemistry Nutrition and Food Science; M. Majewsky, Karlsruhe Institute of Technology (KIT) / Engler Bunte Institut Chair of Water Chemistry and Water Technology; M. Delay, Karlsruhe Institute of Technology KIT; M. Llopart, C. Jares, University of Santiago de Compostela / Department of Analytical Chemistry Nutrition and Food Science; H. Horn, Karlsruhe Institute of Technology KIT / Chair of Water Chemistry and Water Technology; M. Lorens, University of Santiago de Compostela / Department of Analytical Chemistry Nutrition and Food Science

Climbazole (CMZ) is an imidazole anti-dandruff agent widely used in daily life in pharmaceuticals and personal care products (PCPs) like lotions, conditioners, and particularly as an active ingredient of antidandruff formulations. These products can contain up to 2.0 % (w/w) of CMZ, equivalent to a concentration of approximately 0.5 mg/L in antidandruff shampoos. Like other PCPs, CMZ is continuously released in batch water and recreational waters reaching the environment due to incomplete removal during waste water treatment. Recently, CMZ was detected in effluents of a conventional General Electric water treatment plant with a concentration of approximately 0.5 mg/L. Furthermore, ecotoxicity studies demonstrated its exceptionally high toxicity toward selected aquatic organisms and terrestrial plants. UV light is an established free-chlorine method for water and waste water disinfection and a developing method for waste water purification, which can be used in combination with strong antioxidants in the so-called Advanced Oxidation Processes (AOPs), such as heterogeneous photocatalysis (UV/TiO₂). Photolytic or photocatalytic treatment of CMZ can lead to the formation of multiple phototransformation products, which might exhibit similar enhanced ecotoxicological potential relative to that of the parent compound. Additionally, it can be expected that photo degradation also occurs in aquatic environments under natural sunlight. Photodegradation studies about CMZ are rather scarce in the public literature was collected regarding the size of microplastics in facial scrubs and bath glitters. In addition laboratory tests were performed on three toothpastes from different brands to investigate the size and amount of polyethylene speckles. The poster describes the methods used for analyses of particles size distribution and chemical confirmation of polymer identity, and will present an overview of the collected data.

TU221
Ecotoxicity and mixture effects of ultraviolet filters toward selected aquatic organisms
D. השנים-Beludo, Environmental Chemistry, S. Diaz-Cruz, IDEA-ASCI / Environmental Chemistry; D. Barcelo, IQAB-CSIIC / Institute for Environmental Assessment and Water Research
UV Filters (UV-F) constitute a heterogeneous group of chemicals used as a protection against the harmful effects of the UV solar radiation. We can find them in a wide range of compounds such as personal care products or as additives in polymeric materials that need protection against sunlight. Nowadays, these emerging pollutants are considered persistent due to their continuous release into the environment [1]. Thus, the study and understanding of these substances in the environment is of major importance because of their potential to bioaccumulate and consequent toxicity, which may have a negative impact on the receiving environment, especially for biota, or even humans [2]. Some studies performed mostly in vitro but also in vivo have shown that many of these compounds can produce adverse effects on reproduction, disrupting the normal development on fishes and rodents [3, 4]. For instance, 4MBC has similar estrogenic effects than those of 17-ß-estradiol in mammals and amphibious species [5]. Despite that, the knowledge on the potential chronic or acute toxicity to biota of these compounds is still scarce [6]. This study aims to determine the EC50 values of the selected UV-F through a series of toxicological assays on three aquatic species (Daphnia Magna, Selenastrum Capricornutum and Vibrio Fischeri) and to explore the potential synergistic effects in UV-F mixtures. The results indicated that PCPs have EC50 values of mg/L, being slightly more harmful in simpler organisms. Mixtures of PCPs presented normally lower toxicities. Acknowledgment - The authors acknowledge the Generalitat de Catalunya (Consolidated Research Group: Water and Soil Quality Unit 2014-SGR-418), References [1] Gago-Ferrero P., Mastroianni N., Diaz-Cruz M.S., Barcelo D., J. Chromatogr. A 1294 (2013)106. [2] Fent K., Zunker A. and Rapp M., Environ. Pollut. 158 (2010) 1817. [3] Weisbord C.J., Zunker A.K., Kunz P.Y. Fent K. Toxicol. Appl. Pharmacol. 225 (2007) 255. [4] Klammer H., Schlecht C. Wuttke W., Schmutzler, C. Gotthardt I., Köhrle J., Jarry H. Toxicology 238 (2007) 192. [5] Kunz P.Y. and Fent K. Aquatic Toxicology 79 (2006) 305. [6] Seeland A., Oetken M., Kiss A., Fries E., Oehmnn J., Environ. Sci. Pollut. Res. 19 (2012) 1781.

TU222
Ecotoxicological evaluation of the insect repellent DEET. Sub-lethal effects on two aquatic insect species
D. Campos, Department of Biology and CESAM; C. Quintaniero; C. Gravato, University of Porto / Laboratory of Ecotoxicology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; J.J. Pestana, CESAM & University of Aveiro / Department of Biology and CESAM

N,N-diethyl-meta-toluamide (DEET) is an active ingredient present in many insect repellents, and due to its effectiveness against insects and arthropods it has been widely detected in different matrices such as effluents, surface water, groundwater and even drinking water. Despite questions on its ecological effects information concerning DEET’s toxicity in the aquatic environment is still limited and focused on acute effects on model species. Our main objective was to assess the effects of DEET on these non-target species using sub-lethal endpoints. For that we have chosen two aquatic insect species, the caddisfly Sericostoma vittatum and the midge Chironomus riparius, both common in Portuguese freshwaters, with an important role in detritus processing in streams and already used in different ecotoxicological assays. C. riparius larvae were exposed to a gradient of concentrations (8, 12, 18, 27 and 40.5 mg/L) of DEET according to OECD guidelines to assess effects on growth, emergence and adult size; vittatum were exposed to a gradient of concentrations (8, 18 and 40.5 mg/L) of DEET to assess effects on feeding behaviour and biochemical responses. Effects on biochemical responses for C. riparius were evaluated over 48 hour exposures in concentrations of 8, 18 and 40.5 mg/L of DEET. The feeding rates of S.vittatum were significantly reduced by exposure to 40.5 mg/L of DEET. DEET exposure reduced C. riparius larvae growth and delayed emergence of adults (LOEC of 18 mg/L). Body size of adult midges was also significantly reduced with exposure to DEET. Concerning the biochemical responses exposure to DEET caused a reduction in AChE activity. Our results indicate that it’s unlikely for environmental concentrations of DEET to cause ecological deleterious effects in aquatic insects. However, and based on DEET mechanism of action indirect effects (such as predator avoidance and foraging) are to be investigated in the aquatic environment. The research will be also discussed based on the sensitivity of the different aquatic insects as well as on the ecological relevance of the different ecotoxicological endpoints.

TU223
TELOSOL IMPAIRS BYSSUS PRODUCTION
R. Pasturig, Biologia; C. Aguisola, University of Naples Federico II / Biologia; S. Aiello, University of Naples Federico II / Biologia; C. Raimondo, University of Naples Federico II / Biologia; P. Creti, University of Salento; L. Esposito, University of Naples Federico II / Biologia; C.M. Motta, University Federico II of Naples / Department of Biology; B. Avallone, University Federico II of Naples Tropical (TCS) is an antibiotic widely used in many consumer products, being a component of personal care products, textiles and plastics. In recent years its release into the aquatic environment has to deal with many uncertainties in relation to fate and effects. However, the large-scale presence and persistence of (micro)plastics are undebated and from a precautionary point of view, actions to reduce their environmental impact are required. Micropolastics are considered as an potential criterion for allowance and enforcement. However, only limited information is publicly available about the actual particle sizes and amounts of micropolastics in rinse-off products. Company information and public literature was collected regarding the size of microplastics in facial scrubs and bath glitters. In addition laboratory tests were performed on three toothpastes from different brands to investigate the size and amount of polyethylene speckles. The poster describes the methods used for analyses of particles size distribution and chemical confirmation of polymer identity, and will present an overview of the collected data.

TU224
Preening with Personal Care Products: A lack of chronic or reproductive
Toxicity with Decamethylcyclopentasiloxane (D5) in Japanese quail
E.M. Mihalich, ER2; J.M. Stafford, Smithers Viscient; K. Kobayashi, N. Suganuma, silicone Industry Association of Japan (SIA)

Decamethylcyclopentasiloxane (D5) is used in personal care products, such as shampoos, skin creams, and deodorants, as well as in industrial applications and as an intermediate in the production of silicone polymers. Due to its high volatility and high Henry's Law constant the majority of D5 vaporizes into the atmosphere either during manufacturing or during sewage treatment. However, with very low water solubility and high log Kow, the presence of D5 in wastewater effluents and ultimately sludge, with subsequent land application, represents a potential route of distribution to the terrestrial environment. In addition to direct exposure to the land-applied sludge, terrestrial vertebrates could potentially be exposed to contaminated soil. However, there is a second mechanism of exposure that involves the uptake into plants or terrestrial invertebrates and/or uptake into aquatic organisms that might be exposed to the chemical in the aquatic environment. Thus, it was of interest to evaluate the effects of D5 on a higher vertebrate that could be exposed to D5 both directly in the environment and indirectly via the food chain. To assess this, Japanese quail (Coturnix coturnix japonica) were exposed to D5 via diet for the 20 weeks, following OECD guideline 206, and effects on adult health, reproduction, and hatchling success were evaluated. No statistically significant differences were detected in adult body weight gain, adult feed consumption, egg production, embryo viability, embryo survival, eggs hatched, hatching survival or eggshell thickness. The No-observed-Effect Concentration (NOEC), based on adult and hatchling body weight, adult feed consumption, eggshell thickness and all upper interrupted eggs was a QSAR studies fundamentally rely on concepts like molecular structure and the corresponding molecular descriptors, which can then be used to determine and predict, through theoretical, statistical and computational approaches, the biological activity or behaviour of different compounds. This work aims at applying QSAR techniques to analyse molecular descriptors of the UV filters currently approved and regulated in the EU and USA, ascertaining their potential to react with chlorine. Density functional theory (DFT) has been used to obtain molecular descriptors such as electronegativity, hardness, softness and electrophilicity index. Geometry optimizations of the investigated molecules were performed using the wB97X-D functional and the 6-31G(d) basis set. Unsupervised and supervised classifications strategies and linear and non-linear calibration techniques will be used in this QSAR analysis. This approach will allow the analysis of interconnecting relations between the filters, as far as their reactivity towards disinfecting agents such as chlorine. Also, it predicts which compounds will less likely undergo degradation, and will therefore be more advantageous as filters, as well as those which will likely degrade more significantly, yielding potentially harmful by-products.
conduct to obtain more detailed information and data on the different uses of BAS in 80 households in a selected study site in Germany. Households were questioned regarding their use of biocidal products, plant protection products, washing and cleaning agents and cosmetics by a standardised questionnaire. Additionally, the products that were present in the households were registered with the help of a barcode scanner. During the interviews emphasis was laid on the use pattern of biocides but also of other product groups (e.g. because of preservatives that were also used in cosmetic products) that might enter the sewage system. To record the BAS used in other regulatory backgrounds. This contribution presents information on the BAS that were found in these households and the usage pattern of biocidal and cosmetic products. Based on these results the emission of BAS from households to influents of sewage treatment plants is discussed. Herewith, the study extends the knowledge on the potential sources of BAS in wastewater and demonstrates how they distribute over the different regulatory areas, which highlights gaps in the current environmental risk assessments.

TU229
IFRA ENVIRONMENTAL STANDARDS: RISK AND HAZARD ASSESSMENT UPDATE FOR 2015
A. Łapczynski, RIFM / Environmental Specialist; D.T. Salvito, Research Institute for Fragrance Materials, Inc. / Department of Environmental Science; M. Vey, IFRA
To assure safety of fragrance ingredients in consumer products, International Fragrance Association expanded the fragrance industry’s self-regulatory safety framework for aquatic risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials’ (RIFM) testing program in coordination with its Expert Panel. To identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both of these fragrance compounds. Their use in consumer products results in their disposal down the drain to become components of domestic wastewater. They exhibit a wide range of structural classes and physical-chemical properties (e.g., Kow, water solubility). The Research Institute for Fragrance Materials (RIFM) published their screening-level framework for aquatic risk assessment 12 years ago (Salvito, Senna and Fedele, 2002). This approach uses quantitative structure activity relationships (QSARs) to model removal during sewage treatment and toxicity to freshwater aquatic organisms. These QSARs are based on a material’s physical chemical properties and its reported volume of use. The resulting model outputs were used to calculate the ratio of Predicted Environmental Concentration (PEC) to Predicted No Effect Concentration (PNEC). Negligible environmental risk is defined by a resultant PEC to PNEC ratio below one. This framework incorporates procedures for using other available information as well as an optimized strategy for generating new data that are needed to refine and improve the risk assessments. The predicted PECs and PNECs were compared with those based upon measured data. RIFM continues to present at SETAC progress made in the risk assessment of fragrance materials through de novo studies, and thereby building an environmental database of persistence, bioaccumulation, and ecotoxicity information that demonstrate the continued safe use of these materials in consumer products. Presented here is a retrospective analysis of 12 years use of this framework.

Leaving the comfort zone. The challenge of measuring nanoparticles in complex matrices (P)
TU231
Development of single particle ICP-MS for the detection of silver nanoparticles in a complex matrix
k. mylonia, C. Toncelli, HCMR / Institute of Oceanography; A. Tsiola, Institute of Oceanography; I. Kalantzi, Hellenic Centre for Marine Research / Institute of Oceanography; P. Pitta, Hellenic Centre for Marine Research Crete / Institute of Oceanography; S. Pergantis, University of Crete / Department of Chemistry; M. Tsapakis, Institute of Oceanography
The expected increase in the number of engineered nanomaterials (ENMs) due to increased production has impelled the development of novel analytical techniques able to detect and characterize them in environmental samples at ng/L concentrations. Single particle ICP-MS (SP-ICPMS) has been presented as a unique tool for detection and characterization of these nanomaterials at such concentration range. This technique is able to provide information about the elemental chemical composition of nanomaterials as well as their number, concentration, size, and the number size distribution. Because its dynamic range can be extended up to the micrometers region, polydispersed systems as well as aggregation or agglomeration processes may be studied. In addition, dissolved forms of the constituent elements of the nanoparticles can be detected and determined. Recently, several groups have worked at the optimization of detection capabilities and output reporting of SP-ICPMS and, as a result, significant advances in the accuracy of the technique have been reported. However, analysis of different matrix types without the need for any sample pre-treatment, which could potentially create artifacts via NP aggregation, dissolution or partial retaining, are still cumbersome due to signal suppression caused by the matrix. In this study, we have developed an analytical methodology suitable for the detection of AgNPs by SP-ICPMS in a seawater. Indeed, modeling studies showed an expected range has been developed and coupled via a makeup flow to a conventional pneumatic nebulizer. Online dilution of the seawater samples flowing at 50 μL/min was achieved by mixing it with a de-ionized water makeup flow at 1 ml/min approximately 2-3 mm before the tip of a conventional pneumatic nebulizer. The introduction of the seawater samples into SP-ICPMS is carried out via a fused silica capillary (100 μm i.d. and 200 μm o.d.) and a 19.7 μl injection loop. In order to know the transport and ionization efficiency, only the reference nanomaterial was taken into account, thereby eliminating the need of using dissolved silver as additional standard reference. The originality of the analytical method opens new perspectives for the correct assessment of fate and transformation of a strong dynamic redox system, such as the silver nanoparticle/dissolved silver one, in seawater matrix at realistic environmental concentrations (i.e. from 200 ppt onwards).

TU232
Transformation and fate of silver nanoparticle in seawater: use of single particle ICP-MS in complex matrix
C. Toncelli, k. mylonia, HCMR / Institute of Oceanography; I. Kalantzi, Hellenic Centre for Marine Research / Institute of Oceanography; M. Tsapakis, Institute of Oceanography; S.A. Pergantis, University of Crete / Chemistry
Silver nanoparticles are of great concern as their production is expected to rise in the following years and very little is known about their fate and transformation at the marine environment. This study was conducted to obtain more detailed information and data on the different uses of BAS in 80 households in a selected study site in Germany. Households were questioned regarding their use of biocidal products, plant protection products, washing and cleaning agents and cosmetics by a standardised questionnaire. Additionally, the products that were present in the households were registered with the help of a barcode scanner. During the interviews emphasis was laid on the use pattern of biocides but also of other product groups (e.g. because of preservatives that were also used in cosmetic products) that might enter the sewage system. To record the BAS used in other regulatory backgrounds. This contribution presents information on the BAS that were found in these households and the usage pattern of biocidal and cosmetic products. Based on these results the emission of BAS from households to influents of sewage treatment plants is discussed. Herewith, the study extends the knowledge on the potential sources of BAS in wastewater and demonstrates how they distribute over the different regulatory areas, which highlights gaps in the current environmental risk assessments.

G. Cornelis, University of Gothenburg / Department of Chemistry and Molecular Biology
Nanocount: user friendly spICP-MS data analysis software
TU233
Nanocount: user friendly spICP-MS data analysis software
G. Cornwall, University of Gothenburg / Department of Chemistry and Molecular Biology

Interest in applying single particle ICP-MS (spICP-MS) in risk assessment of inorganic engineered nanomaterials (ENM) has been increasing because it is currently the only technique capable of measuring number-based particle size distributions of ENM at environmentally relevant number concentrations in complex environments. However, the large size of spICP-MS datasets makes data treatment cumbersome, even though the basic spICP-MS calculations are relatively straightforward. Most of the advanced data analysis methods and algorithms for deconvolution are required to measure the lowest sizes possible where considerable overlap between dissolved and particulate signals exists. The application of such algorithms requires software. NanoCount© is a spICP-MS data treatment software platform that can accept data from any ICP-MS off-line and offers large flexibility in currently available data-treatment algorithms such as n x sigma, K-means clustering and density clustering. To distinguish dissolved and particulate signals in spICP-MS datasets. In addition, it allows different approaches to calculate nebulisation efficiency and NanoCount© also has many options to account for signal drift, because spICP-MS requires extremely stable signals. Finally, many options are available to customize the calculated particle size distributions. While software such as NanoCount may increase accessibility of spICP-MS, it is demonstrated in the current paper using spICP-MS data from 15 nm Au ENM, how different data-treatment approaches in spICP-MS can lead to different results. Current validation of spICP-MS analysis focuses on experimental protocols and finds wide variability in the obtained results. It is argued here that a large portion of this variability can be explained by differences in data treatment. There is thus a need for data treatment validation and standardisation, in addition to standardisation of the experimental approach.

**TU234**

Development of an UHPLC-QTOF MS method for the analysis of fullerenes in soil and sediment matrices
A. Carboni, IBED; R. Helmus, University of Amsterdam; J. Parsons, K. Kalbitz, P. de Voogt, University of Amsterdam / IBED

Fullerenes are carbon based nanoparticles receiving attention for their potential use in nanotechnology and their increasing production and application is cause of concern for both toxicological and environmental aspects. To date, data reporting their occurrence in the environment are scarce and often contradicting. Possible pathways for their release are expected to be 1) the direct release from materials that contain them, 2) unintentional production (e.g. by-product of combustion processes). In addition, natural processes may account for their presence in the environment such as, and few methods are available for the analysis of emerging contaminants in environmental matrices. Most of the research focused on aqueous compartments (e.g. surface and ground water) more than in soil and sediments. Furthermore, most of the analytical methodology available are characterized with high detection limits and are not suitable for the analysis of expected trace levels within the environment. In this study, we developed a method for the analysis of fullerenes in soil and sediment matrices. Fast chromatographic separation with UHPLC (Rt=1.5) was achieved with the use of biphenyl stationary phase whereas detection with Ion-Booster-Q-TOF high resolution mass spectrometry granted high sensitivity and mass resolution. The detector response showed high linear dynamic range with detection and quantification limits of 0.6 ng/L and 1.9 ng/L respectively. The developed method was then validated for the analysis of fullerenes spiked into soil and sediments extracts (n=6) with differing properties such as texture and organic carbon content. Particular attention was paid to the matrix effect and post-column infusion and standard addition experiments showed that the method is suited for the analysis of the majority of the matrices included in the present study. Furthermore, signal suppression in very complex matrices (e.g. high carbon concentration) could be handled with a simple modification of the mobile phase composition. Although fragmentation could not be obtained for the fullerenes, the application of high collision energy provided a “background clean-up” that resulted in extremely clean and reliable MS² spectra, where only the precursor ion was visible, in all the samples. Finally, it was shown how the method is suitable for the analysis of other fullerenes such as functionalized derivatives.

**TU235**

Environmental survey of fullerenes in urban and industrial soils
A. Carboni, IBED; R. Helmus, University of Amsterdam; J. Parsons, K. Kalbitz, P. de Voogt, University of Amsterdam / IBED

Fullerenes are considered as some of the most promising materials in nanotechnology and their increasing production and application is cause of concern for both toxicological and environmental aspects. To date, data reporting their occurrence in the environment are scarce and often contradicting. Possible pathways for their release are expected to be 1) the direct release from materials that contain them, 2) unintentional production (e.g. during combustion processes) and 3) natural processes. Furthermore, most of the research focused on aqueous compartments (e.g. surface and ground water) more than in soil and sediments. The samples were collected in different areas across the Netherlands. The samples were processed and analyzed with a recently developed method that allowed the detection at concentrations in the range of the expected environmental concentrations (i.e. ng/kg). C60 fullerene was detected in samples from different locations whereas C70 was not present in any of the samples analyzed. In addition to C60, one product of transformation that preserves the C60 cage was identified in few of the samples analyzed. These data will contribute to understanding the sources and pathways of contamination by fullerenes in soils.

**TU236**

ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION HYPERENHANCED TO ORBITRAP HIGH RESOLUTION MASS SPECTROMETRY FOR THE DETERMINATION OF (FUNCTIONALISED) AQUEOUS FULLERENE AGGREGATES
P.S. Bauerlein, KWR / Analytical and Environmental Chemistry; E. Emke, KWR Watercycle Research Institute; P. de Voogt, University of Amsterdam / IBED; P. Herrera, E. Pocurull, Universitat Rovira i Virgili

We report on the technical implementations of coupling an asymmetric flow field-flow fractionation (AF4) instrument to a high resolution mass spectrometer (Orbitrap) using an atmospheric photoionisation interface [1]. This will allow for the first time an online identification of different fullerenes in aqueous samples. After their aggregates have been fractionated in the FFF channel. It is now possible to analyse fullerene aggregates firstly with non-destructive techniques such as UV-Vis or MALDI and subsequently use MS to identify and quantify the fullerenes. Quality parameters such as limits of detection (LODs), limits of quantification (LOQs) or linear range for the mass spectrometer were evaluated and they were in the range of hundreds ng/L for LODs and LOQs and the detector response was linear in the range tested (up to ~ 20 µg/L). Crucial for this setup are the use of the slot pump to reduce the flow to the detectors as well as the gas dopant device, which introduces toluene. Toluene is needed for enhancement of the ionisation of fullerenes. The low detection and quantification limits make this technique useful for future environmental or ecotoxicology studies in which low concentration levels are expected for fullerenes and common organic pollutants, such as PAHs, are expected for fullerenes and common organic pollutants, such as PAHs, in complex environments. However, the large size of spICP-MS datasets. In addition, it allows different approaches to calculate nebulisation efficiency and NanoCount© also has many options to account for signal drift, because spICP-MS requires extremely stable signals. Finally, many options are available to customize the calculated particle size distributions. While software such as NanoCount may increase accessibility of spICP-MS, it is demonstrated in the current paper using spICP-MS data from 15 nm Au ENM, how different data-treatment approaches in spICP-MS can lead to different results. Current validation of spICP-MS analysis focuses on experimental protocols and finds wide variability in the obtained results. It is argued here that a large portion of this variability can be explained by differences in data treatment. There is thus a need for data treatment validation and standardisation, in addition to standardisation of the experimental approach.

**TU237**

Analytical challenges in identification of nanometer plastics
S.A. Kools, KWR Watercycle Research Institute; P.S. Bauerlein, KWR / Analytical and Environmental Chemistry; P. Koosj, KWR Watercycle Research Institute; P. de Voogt, University of Amsterdam / IBED; W. Siegers, KWR Watercycle Research Institute

Plastics fragments may affect life and analysis of particles is of the attention of many. To underpin research on sources of plastic fragments, our research aims to detect and identify plastics in water matrices. We focus our work on the smaller scale fragments as low as 1 µm in size. In this study, we developed a method for the analysis of plastics in water matrices. We demonstrate that for fullerenes and common on environmental or ecotoxicology studies in which low concentration levels are expected for fullerenes and common organic pollutants, such as PAHs, in complex environments. However, the large size of spICP-MS datasets. In addition, it allows different approaches to calculate nebulisation efficiency and NanoCount© also has many options to account for signal drift, because spICP-MS requires extremely stable signals. Finally, many options are available to customize the calculated particle size distributions. While software such as NanoCount may increase accessibility of spICP-MS, it is demonstrated in the current paper using spICP-MS data from 15 nm Au ENM, how different data-treatment approaches in spICP-MS can lead to different results. Current validation of spICP-MS analysis focuses on experimental protocols and finds wide variability in the obtained results. It is argued here that a large portion of this variability can be explained by differences in data treatment. There is thus a need for data treatment validation and standardisation, in addition to standardisation of the experimental approach.

**TU238**

Applying a generic sample preparation approach to isolate nanomaterials from food and cosmetics
M. Velimirovic, University of Vienna / Department of Environmental Geosciences; S. Werner, University of Vienna / Department of Environmental Geosciences; T. Hofmann, University of Vienna / Department of Environmental Geosciences; P. Von der Kammer, Vienna University / Department of Environmental Geosciences; T. Hofmann, Vienna University / Department of Environmental Geosciences

The applicability of a generic scheme to systematically develop methods for detection, characterization, and quantification of engineered nanoparticles (ENPs) and nanomaterials (NM) in food and cosmetic products is tested. We select two sample types and applied the generic sample preparation scheme: 1) a powdered tomato soup which contains the anti-caking agent SiO2 (ES551), and 2) a sunscreen which contains TiO2 as UV-filter. Our research focused on sample preparation, aiming to achieve a complete separation of ENPs from the respective matrix without altering the ENP size distribution and with minimal loss of ENPs. The generic multi-step sample preparation process includes: I) homogenisation of the sample; II) ENP separation from the matrix; III) ENP enrichment if necessary, and IV) ENP stabilization. The preparation procedure was evaluated by pre-defined quality criteria, which are mass recovery and change in size distribution. The mass recovery was calculated by elemental analysis of Si and Ti, respectively. The size distribution of the isolated ENPs was determined by asymmetric flow field-flow fractionation (AF4) coupled to multi-angle laser light scattering (MALLS) and inductively-coupled plasma mass spectrometry (ICP-MS). The size distribution was compared to the size distribution of pristine particles. We demonstrated that for both sample types the generic sample preparation scheme is valid. For SiO2 in tomato soup, complete matrix removal and Si mass recovery > 90% was achieved.
using acid digestion supported by heat (90°C) and hydrogen peroxide as oxidation
reagents. Acid digestion supported by heat (90°C) and hydrogen peroxide as oxidation
reagents is important for the characterization of nanomaterials and
to amend and develop new test guidelines specifically for nanomaterials. Until then,
etcotoxicity testing of NMs in order to
classification as nanoparticles. DLS analysis were performed using a Couduorn
Technology VASCO-2 particle size analyzer. The original ink sample (7.3 mg/mL)
was diluted in water: 1/10, 1/100 and 1/200. Polidispersity index (PDI) has been
calculated according to the Cumulants method (Koppel, 1972; Fiskin, 2001), being
0.2960 (for 1/10 dilution), 0.1320 (for 1/100 dilution) and 0.02120 (for 1/200
dilution). Size distribution was obtained taking into account the number of the particles
and their volume (nm). Results showed that 10% of the particles have a diameter less
than 181±20 (CV=11%) nm and 90% of the particles have a diameter less than
211±18 (CV=8%) nm. Similar results were obtained and the majority of the particles are in a range of 100 to 200 nanometers, but with the DLS
the size is a bit larger. This may be because the diameter determined by DLS is a
hydrodynamic size, and is larger than the dry particle size. Bibliography: Jiang

TU239
PIlot study about the behavior of different sized noble metal nanoparticles in
Daphnia magna and related exposure media
S. Brandaña, Institute for environmental studies (IVM); P. Krystek, MiPlaza, Philips
Research Europe; P. Leonards, VU University, Institute for Environmental Studies /
Institute for Environmental Studies IVM
As a new approach, exposure scenarios with different noble metal nanoparticles
were setup. The particles are chosen based on their elemental composition (silver,
gold and platinum) and the particles sizes of 5 to 50 nm i.d. Daphnia magna was
exposed for a period of 24 hours. To localize the nanoparticles a microEDX technique
was used. EDX analysis of the Daphnia gut samples revealed that nanoparticles were present in the body. The uptake in
Daphnia and the nanoparticle stability in the exposure media were studied. Various
analytical approaches were evaluated and presented in detail.

TU240
Development of a ‘best practice’ for algae testing of nanomaterials based on
available recommendations from the scientific literature and other available
guidance
A. Brinch, DTU Technical University of Denmark / Department of Environmental
Engineering; S.F. Hansen, Technical University of Denmark DTU / DTU
Environment; N.B. Hartmann, Technical University of Denmark (DTU) / DTU
Environment
Ecotoxicological data is a fundamental information requirement in different
chemical legislations such as the European chemical legislation, REACH and the
Biocidal Products Regulation (BPR). However, standard test guidelines for the
ecotoxicity testing of NMs are lacking. Work is ongoing within the OECD WPMP to
toxicity guidelines specifically for nanomaterials. Until then, however, registrants and regulators must rely on currently available guidance and recommendations from other sources. Here we collect and analyse this readily available information from various sources including scientific literature and
OECD guidance documents. On this basis we suggest a best practice for how to
carried out appropriate ecotoxicity testing of NMs in order to provide reliable data for the
activity.
EDX analysis of the Daphnia gut samples revealed that nanoparticles were present in the body. The uptake in
Daphnia and the nanoparticle stability in the exposure media were studied. Various
analytical approaches were evaluated and presented in detail.

TU241
Characterization of sepiak ink eumelanins through SEM and DLS
D.E. Gomez, The University of Exeter / College of Engineering Mathematics and
Physical Sciences; M. Paradelo, University of Vigo / Plant Biology and Soil
Science; V. Pérez, Universitat de Vigo / Plant Biology and Soil Science; J.J. Piriápolis, University of Vigo / Plant Biology and Soil Science; I. De La Calle,
University of Vigo / Analytical and Food Chemistry
Eumelanins are black and insoluble pigments that give dark hue to several organic
pigments. They are in the upper limit of the
indirectly probe the GO adsorption capacity by looking at revertants induction caused by non-adsorbed testing molecules (pollutants) on GO surfaces. We selected an important mutagenic pollutant produced from incomplete combustion of fossil fuels (3-nitrobenzanthrone (3-NBA)) as the testing molecule. We observed that GO can adsorb 3-NBA and this adsorption is dependent on the amounts of OD on the GO surface used in the test, which indicates that OD indeed reduce the GO adsorption capacity. Therefore, we found that GO can selectively adsorb 3-NBA from wastewater where GO-Ag L2 samples, which has around 50% less OD than GO-Ag-L4, were 20% more effective than GO-Ag-L4 samples. While the presence of OD on the GO surface is interesting to increase the GO stability in water, the OD inhibit stronger interactions between 3-NBA and GO. Our data strongly indicate that OD are important molecules on the GO structure that should be taken into account in future studies. The authors acknowledge São Paulo Research Foundation FAPESP (2012/1982-5) for financial support.

TU244
Comparative evaluation of methods to quantify dissolution of nanomaterials
N.B. Hartmann, Technical University of Denmark (DTU) / DTU Environment; S. Kruse, DTU Environment and Department of Environmental Engineering

Effects and behaviour of nanomaterials in the environment depends on the materials’ specific physical and chemical properties and for certain nanomaterials (e.g., Ag, ZnO and CuO) aqueous solubility is of outmost importance. The solubility of metals salts is normally described as a maximum dissolved concentration or by the ion equilibrium constant. For Cu ions it is essential to also assess solubility kinetics as nanomaterials will often not dissolve instantaneously upon contact with artificial aqueous media or natural waters. Disolution kinetics will thereby influence their short and long-term environmental fate as well as laboratory test results. This highlights the need to evaluate and improve the reliability of methods applied to assess the solubility kinetics of nanomaterials. Based on existing OECD guidelines and existing documents on aqueous dissolution of metals and metal compounds, the aim of this project was to conduct systematic experiments contributing to a better understanding of how nanomaterials solubility can be tested and evaluated under the auspice of the OECD framework. The focus was to evaluate the performance of three different techniques used to separate the dissolved and non-dissolved fraction of nanomaterials, namely: dialysis membranes (MWCO 8-10 and 50 kD), Diffusive Gradients in Thin films (DGT) and ultracentrifugation. The release of Cu ions from CuO nanoparticles (primary nominal size <50nm) was used as a case study. Micron sized CuO was used as a “non-nano reference” and CuSO4 and CuCl2 were used as ion references. In a comparative study of the three methods we found a good reproducibility between replicates (ranging from 99% to 101% of nominal concentrations). However, we observed a large difference in results between the three applied methods. Preliminary results showed that by using the DGT method the measured dissolved fraction was 5-7 times higher compared to using ultracentrifugation and dialysis membranes. Possible explanations include metal-specific interactions with the dialysis membranes as well as the DGT unit including the potential contribution to the low recovery step for changing in behaviour of larval feeding habits during the development time or possibly after 10 d time larvae have reached steady stage. Using the exposure method 2, larval growth and emergence rate were affected by fullerenes. Findings indicated that presence of fullerene interferes organisms even at the lowest tested method (2, 0.5 mg/kg) concentration. Overall, these results pose that there is possible ecotoxicity towards benthic organisms that fullerene can cause leading changes in ecotoxic parameters used here.

TU245
Aggregation dynamics of silver nanoparticles in ecotoxicological test media
G. Metreveli, Institute for Environmental Sciences Group of Environmental and Soil Chemistry; B. Frombold, Universität Koblenz

Aggregation dynamics and mechanisms of silver nanoparticles (Ag NPs) for different conditions were investigated. A partial dissolution of Ag NPs was observed in all media in absence, as well as in presence of NOM. The release of silver ions from Ag NPs was controlled by the interplay of protecting mechanisms and stabilizing effects of NOM, as well as by complexation and re-sorption of released ions. In each TM, Ag NPs aggregated in a reaction-limited regime. The aggregation mechanisms and dynamics were controlled by the ion composition, presence of specifically interacting organic compounds (vitamins, proteins), NOM quality and concentration of Ag NPs. Electrostatic stabilization by NOM was most effective at low Ca2+ and Mg2+ concentrations. At high Ca2+ and Mg2+ concentration, NOM accelerated aggregation due to formation of bridging-determined aggregates. Bridging-determined aggregation was much more prominent. Aggregation of Ag NPs was influenced by the composition of the TM, concentration of Ag NPs, and the duration of the ecotoxicological tests can affect the dissolution and aggregation status of Ag NPs. These findings underline the requirement to carefully interpret associated ecotoxicological data. The results obtained in this study can also be used for tuning the aggregation and coating properties of Ag NPs in different TM. For example, the results of the Ca2+/Mg2+ ratio in ranges acceptable for the organisms will reduce the aggregation rate of nanoparticles. The further amendment of TM with NOM will result in different types of colloidal stabilization. The addition of specifically interacting organic compounds like vitamins or proteins may further strengthen the organic coating.

TU246
FullereC60 loading interferes of the growth and emergence rate of the Midge Chironomus riparius
G.C. Waisi-Leinonen, University of Eastern Finland; I. Nybom, Department of Biology; K. Pakarinen, University of Eastern Finland / Department of Biology; F. Petersen, National Institute of Standards & Technology; J. Akkanen, University of Eastern Finland / Department of Biology; M.T. Leppanen, Finnish Environment Institute / Laboratory Centre: J.V. Kukkonen, University of Jyväskyla / Biological and Environmental Science

The suitability of test methods for use with nanomaterials is a topic of significant research interest. There are several uncertainties when assessing nanomaterials, such as fullerene(C60), potential toxicity to aquatic invertebrates because of their uncharacterized physical properties and ecotoxicological impacts. The aim of this study was to investigate the method for measuring the body residues on Chironomus riparius and to study if sediment associated fullerene had an impact on larval development and emergence rate. A benthic invertebrate C. riparius larvae were exposed to nC60, with two different exposure methods: 1) masses in the sediment top layer creating an environmentally realistic method and simultaneously a sensitive exposure route for C. riparius feeding habits and 2) using fulleren spiked sediment which is commonly used method in ecotoxicology. Body residues after acute and chronic exposures were analysed and larval growth and development rate assessed. Body residues were lower in the exposure method 1 after 15 d exposure than after 10 d which may stem for changing in behaviour of larval feeding habits during the development time or possibly after 10 d time larvae have reached steady state. Acute exposure studies indicated that C. riparius is a ‘sink’ for fullerenes. These findings underline the requirement to carefully interpret associated ecotoxicological data. The results obtained in this study can also be used for tuning the aggregation and coating properties of Ag NPs in different TM. For example, the results of the Ca2+/Mg2+ ratio in ranges acceptable for the organisms will reduce the aggregation rate of nanoparticles. The further amendment of TM with NOM will result in different types of colloidal stabilization. The addition of specifically interacting organic compounds like vitamins or proteins may further strengthen the organic coating.
TU248 Evaluation of environmental toxicity for surface-modified silver nanoparticles on aquatic organisms

Y. Jung, Convergence Environment Team; S. Baik, KIST Europe / Convergence Environment Team; C. PARK, Korea Institute of Science and Technology Europe / Convergence Environment Team Environment Bio Group; S. Kim, KIST Europe / Chemical Management Lab

With the increased use and application of surface modified silver nanoparticles (AgNPs), many concerns have been raised for their toxicity after releasing and discharging to aquatic ecosystems which may pose possible risks to the environment. Therefore, in this study, ecotoxicological evaluation of surface-modified AgNPs on aquatic organisms was conducted. Aquatic organisms for the ecotoxicological evaluation were luminescent bacteria and Daphnia magna, and four different surface coated AgNPs with the same core diameter of about 20 nm were used. Surface coating materials for AgNPs were citrate (Cit), tannic acid (Tan), polyethylene glycol (PEG), and branched polyethyleneimine (BPEI) and ζ potentials of those NPs were -27.1 mV, -10.7 mV, -20.5 mV, and 18.5 mV, respectively. For an acute (15 min) ecotoxicological screening test, luminescent-microarray was used for Daphnia magna, which consists of 2 fresh water and 9 marine bacteria, was applied. The tendency of toxicity effects for target AgNPs on the marine bacteria are following order: BPEI-AgNPs > PEG-AgNPs > Tan-AgNPs > Cit-AgNPs, resulting on the positively charged AgNPs with higher salt stability showed more toxic on those marine luminescent bacteria. The other acute (48h) AgNPs toxicity test was conducted in Daphnia magna by monitoring Na/K ATPase activity of their respective tissues of Potamonautes warreni.

TU249 The eco-corona mediated uptake and impacts of so-called “Future Nanomaterials” on Daphnia magna

F. Nasser, University of Birmingham / Geography Earth and Environmental Science; E. Valsami-Jones, The Natural History Museum; I. Lynch, University of Birmingham / Geography Earth Environment Science Nanoparticles (NPs) can be defined as having at least one external dimension between 1-100nm. Due to their small size, NPs have a large surface area to volume ratio which gives them unusual properties that differ from bulk material of the same chemical composition. As a result these novel materials have found numerous applications in areas such as drug delivery, medicine and electronics. Previous research has been conducted on legacy NPs such as silver which acts as an antimicrobial agent; gold which is used in cancer therapy and; carbon nanotubes which are widely used in the electronics industry. The future of nanotechnology is promising, however the presence of nanoparticles in the aquatic environment has raised environmental concerns as the exact potential of the different generation NPs are not yet known. In order to understand the potential threats caused by nanoparticles it is important to study their interactions with aquatic organisms. In this study, the interactions of nanoparticles on Daphnia magna were assessed using gold NPs of a range of morphologies (rods, spherical or rod-like) and a variety of surface coating materials. The results suggested that the antioxidant defences of D. magna were significantly affected by exposure to exposure to environmentally-relevant concentration of AgNPs.

TU251 Absorption and distribution of ingested silica nanoparticles in Blattella germanica: a potential carrier in the development of nanoparticles

I. Biagnoni, M. Ochoa-Zapater, Universitat de Valencia / Dpt Biología Funcional y Antropología Física; A. Ribera, Universitat deValencia / Instituto de Ciencia Molecular; A. Torrelbana, University of Valencia / Department of Animal Biology; F. Romero, Universitat de València / Dpt Química inorgànica; L. Varo, Spanish Research Council (CSIC) / Biology culture and pathology of marine species; M. Garcerá, Universitat de València / Dpt Biología Funcional y Antropología Física

The rapid advancements in nanotechnology have provided a wide range of nanomaterials that have unique properties that open new possibilities of application in medicine, agricultural productivity and pest control. Nanoparticles can be used in agriculture as insecticide carriers in order to improve efficiency, reduce environmental pollution and the impact on non-target organisms of conventional pesticides. Mesoporous silica nanoparticles could be promising candidates as carriers due to their high surface area and the porosity properties that allow the storage and controlled release of active agents. We investigate absorption, penetration and distribution of mesoporous silica NPs. The agents tested were the fluorophore rhodamine B, a dye for visualizing and tracking the nanoparticles and the insecticide permethrin, a pyrethroid, a compound often used in pest control. In the first instance, the potential penetration of the nanoparticles was determined by oral ingestion. Later insect test were conducted, allowing us to evaluate the potential for the nanoparticles to be adsorbed in the digestive system and subsequently increase the pesticide efficacy.

TU252 Effect of freshwater algal growth stage on physicochemical properties, transformations, and toxicity of iron nanoparticles

A.S. Adelwage, University of California, Santa Barbara / Bren School of Environmental Science and Management; M.W. Shively, Ecology Evolution and Marine Biology; Y. Su, Tongji University / State Key Laboratory of Pollution Control and Resources Reuse; R.M. Nisbet, UCSB / Ecology Evolution and Marine Biology; A.A. Keller, Bren School of Environmental Science and Management

A number of pilot and full scale field studies have shown that nanoscale zero-valent iron (nZVI) and its derivatives are promising for remediation of several important environmental pollutants. However, there is still a lot of uncertainty about the implications of injecting high concentrations of these engineered nanomaterials (ENMs) into the natural environment. The implications of ENMs in the environment are controlled mainly by the ENMs’ physicochemical properties, stability, transformation, and the bioavailability of their different transformation products (TFPs). In this study, the physicochemical properties of two iron nano zero-valent iron (nZVI) (FeSsI) were compared in both fresh algal media (Day 1 media) and media in which Chlamydomonas reinhardtii had been grown for 11 days (Day 11 media) in order to elicit the effect of organic matter produced by the freshwater alga. In addition, dissolution and transformation of FeSsI particles were investigated for a month in Day 1 and Day 11 media inoculated with C. reinhardtii, while monitoring the changes of both set of TFPs, resulting from the exposure to nZVI for 11 days. The results showed that the growth stage of algae at which they are exposed to ENMs may have a strong effect on the physicochemical properties, transformations, and toxic effects of the particles.
DNA changes in Pseudomonas putida induced by zirconium oxide nanoparticles using RAPD analysis

M. Zalecka-Radziewill, Warsaw University of Technology / Department of Biology; N. Doskocz, Warsaw University and Technology / Department of Biology

Unique properties of nanoparticles, such as, among others, their small size, a large surface area, high chemical reactivity, may result in profound contamination by nanotechnology extremely attractive compared to those obtained by traditional methods. With the arrival of nanomaterials and their widespread application there is a great risk of exposure of organisms in ecosystems to this type of contamination. Research has been conducted to determine the genotoxicity of zirconium oxide nanoparticles towards Pseudomonas putida using RAPD-PCR method (Random Amplification of Polymeric DNA). The results were compared with the effects of the impact of the macro form of the compound on DNA. The PCR reaction used the following primers: OPA 2, OPA 10, OPA 9 and OPA 18 of a random nucleotide sequence. On the basis of RAPD profiles using primer OPA 2 and OPA 10 the research demonstrated mutagenic action of the tested nano-compound. The obtained profiles of RAPD bands are different from the negative control of filtrates indicating an important desorption even in freshwater. Similarly, when clay particles with already adsorbed AgNP are added to river and brackish waters, between 75 to 90% of labelled AgNP is recovered in filtrates indicating an important disadvantage of AgNP going through filter and/or a dissolution process during the 24th adsorption period. These data are being incorporated in a model of the environmental fate of AgNP at the river/ocean front.”

TU255
Effects of different feeding media and nano-TiO2 particles on the food uptake of Caenorhabditis elegans

K. Samet, HAW- Hamburg / Medical Engineering; J. Angelstorf, Hamburg University of Applied Sciences; S. Heise, Hamburg University of Applied Sciences / Life Sciences

Even though the amount of industrially manufactured nano-particles used in e.g. consumer products has been increasing steadily over the last years, information on ecotoxicity is limited. Simulating the accumulation of nano-scale titanium dioxide (n-TiO2) in rivers, Gottschalk et al. (2009) calculated an accumulation of 1.4 mg / m2 in coastal sediment. This is 1000 times higher than the accumulation of other nano-materials. Lowest observed effect concentration of n-TiO2 to reproduction of C. elegans was 10 mg / L n-TiO2, agglomerated in the nematode’s intestine in the absence of bacteria. Bacterial cells showed a potential protective function to the intestinal cells of C. elegans against accumulation with n-TiO2. A Daphnia daphnia was observed as indicated by the uptake and transport of microspheres along the intestine (Angelstorf et al. 2014). This study focuses on the mode of action of n-TiO2 during exposure of the nematode Caenorhabditis elegans and the discrimination between the effect of bacterial cells and their extracellular polymers on n-TiO2 in the nematode’s intestine. Results of different feeding experiments, indicating the effect of different combinations of n-TiO2, exopolymers and bacterial cells on the uptake, agglomeration and toxicity of n-TiO2 could help to elucidate one mode of action of nano-scale titanium dioxide in organism. Following the standardized bioassay ISO 10872 with n-TiO2 in a concentration of 100 mg / L as test-material and different feeding experiments with the bacteria Escherichia coli resulted in digestive problems observed by the uptake and transport of microspheres as indicator for the digestion process in the nematode’s body. Digestive problems and growth inhibition were observed in the test-organism during exposure to n-TiO2 with and without extracellular polymers as feeding medium. Feeding nematodes with extracellular polymers caused also an inhibition in growth compared to the nematodes fed with bacterial cells. As indicated by the uptake of microspheres the attenuation of n-TiO2 agglomeration in the intestine of C. elegans was not caused by bacterial exopolymers but probably by bacterial cells.
The influence of soil properties on particle fate of silver nanoparticles and their toxicity to C. elegans and E. coli

C. van Gestel, VU University Amsterdam / Department of Ecological Science; A.M. Soares, Universidade de Aveiro / Department of Biology CESAM; C. Svendsen, CEH, Wallingford / Pollutio and Ecotoxicology

Due to their high reactivity manufactured nanoparticles will likely undergo various transformations after entering the environment. Such transformations will affect their fate and behaviour, such as dissolution, agglomeration and sedimentation, which can in turn influence their bioavailability and resultant toxicity. It is therefore important to consider the effect of environmental properties on nanoparticle chemistry and form to understand how exposure relevant particles are presented to, and taken up by, organisms. The effect of soil properties on the toxicity of two silver nanoparticles (AgNPs) to C. elegans and E. coli strain OP50 was investigated by performing exposures in pore waters extracted from soils of different chemistry. Soil properties assessed were soil organic matter content (4.18% vs. 16.70%) and pH (pH 4.8 vs. 6.1 vs. 7.2) and the particles used were a small 3-8 nm un-functionalised AgNP (3-8Ag) and a larger 52 nm PVP-coated AgNP (52PVP). Effects on the nanoparticle agglomeration state and stability over time was assessed using asymmetric field flow fractionation (AFF) and UV/vis absorbance measurements. Nanoparticles were exposed as gravid adults to a range of concentrations of the particles as well as ionic silver for 72 h and their reproductive toxicity was subsequently assessed. The results of the nematode toxicity tests showed ionic silver had a higher toxicity than the tested AgNPs, with EC50 concentrations being at least a magnitude lower across all the tested pore waters, whereas there was little difference in toxicity between the two AgNPs and no distinct trend between soil pH levels. Increasing the organic matter content significantly decreased the toxicity of 3-8Ag while it increased that of AgNO3. Soil pH of the extracted pore waters significantly changed the toxicity of ionic and both silver nanoparticles, with highest toxicity observed at the lowest pH, for both AgNPs where there was a 10 fold decrease in EC50 concentration. The bacterial toxicity testing is still ongoing. Nanoparticle characterisation with UV/vis spectroscopy revealed only a slight agglomeration dynamics independent of soil properties for 3-8Ag, while higher organic matter and low pH appeared to stabilise the 52PVP particles in suspension. Overall both soil organic matter content as well as pH had a nanoparticle dependent effect on fate as well as toxicity to C. elegans, however, there appears to be no clear connection between the measure particle properties and their effect.

The Effects of Silver Nanoparticles and Silver Nitrate on Plants, Collemboila and Earthworms in Soil

J. Velicogna, Biological Assessment and Standardization Section; J. Prince, Environment Canada; S. Detti, University of Rome Tor Vergata / Department of Business Engineering Mario Lucertini; R. Pini, CNR / Institute of Ecosystem Studies; A. Bianco, National Research Council of Italy / Institute of Ecosystem Studies; A. Marzese, CEH, Wallingford / Pollutio and Ecotoxicology

A suite of soil toxicity tests were completed to examine and compare the effects of commercially available 20 nm PVP (0.3%) coated silver nanoparticles (AgNP) to ionic silver (Ag+, as AgNO3) in a sandy-loam field soil. Plant tests examined effects on net primary productivity and growth of Elymus lanceolatus and Trifolium pratense. Invertebrate tests examined reproduction and survival of Folsomia candida and Eisenia fetida, as well as bioaccumulation for E. andrei. Elymus lanceolatus, T. pratense and F. candida were more sensitive to the Ag+ exposures, relative to the AgNP for all measured endpoints. However, effects on juvenile production and dry mass of E. andrei were comparable upon exposure to both Ag+ and AgNP, and were the most sensitive test organisms in general. For example, E. andrei reproduction was significantly affected when exposed to 249 mg/kg AgNP, whereas the EC50 was 29 mg/kg Ag+ (when exposed to AgNO3). When considering bioaccumulation metrics, for both forms of Ag (AgNP and Ag+), a rapid uptake was observed in earthworms when exposed to 4 mg/kg Ag during the first week of the uptake phase. The bioaccumulation factor at the end of the uptake phase (i.e., day 21) was 0.71 and 0.80 for AgNO3 and AgNP, respectively. Silver in tissue remained significantly higher than background levels throughout the entire phase with 48 and 96% remaining in the AgNO3 and AgNP exposed worms, respectively. Transmission electron microscopy (TEM) confirmed that AgNP were present in tissue at the end of the 21 day uptake phase. Further efforts will be needed towards the evaluation of AgNP in biological tissue, and will include a comparison and evaluation of AgNP when added to soil through the application of contaminated biosolids, the route through which AgNP are likely to enter into the environment.

Toxicokinetics and Toxicodynamics of Silver Nanoparticles in Enchytraeus crypticus

E. Topuz, VU University Amsterdam / Department of Ecological Science; C. van Gestel, VU University Amsterdam / Ecological Science

Silver nanoparticles (AgNPs) are used in numerous consumer products and may end up in ecosystems, especially in sediments due to agglomeration over time, depending on surface water properties. AgNPs that enter wastewater treatment plants mostly partition in the sewage sludge fraction during treatment. Since in many countries sewage sludge is applied to soil it is urgent to investigate effects of AgNPs on soil organisms. Investigation of the relationship between the toxicity and internal concentration of organisms might be more beneficial since external concentration is affected by uptake, toxicity and excretion [8]. Enchytraeids are essential for the functioning of the ecosystem and sensitive to the numerous stressors [5], with Enchytraeus crypticus being a suitable model for soil ecotoxicity testing [6]. The aim of this study is to investigate the toxicokinetics and toxicodynamics of AgNPs in Enchytraeus crypticus. By performing experiments in water and soil, results will provide insight on the role of porewater in the exposure of soil nanoparticles to Enchytraeus crypticus [9]. Enchytraeids are exposed to the AgNP and AgNO3 in different soils and by measuring Ag concentrations in the animals, results of solution and soil uptake are linked to soil properties. Nematodes were exposed as gravid adults to a range of concentrations of the particles as well as ionic silver for 72 h and their reproductive toxicity was subsequently assessed. Results of the nematode toxicity tests showed ionic silver had a higher toxicity than the tested AgNPs, with EC50 concentrations being at least a magnitude lower across all the tested pore waters, whereas there was little difference in toxicity between the two AgNPs and no distinct trend between soil pH levels. Increasing the organic matter content significantly decreased the toxicity of 3-8Ag while it increased that of AgNO3. Soil pH of the extracted pore waters significantly changed the toxicity of ionic and both silver nanoparticles, with highest toxicity observed at the lowest pH, for both AgNPs where there was a 10 fold decrease in EC50 concentration. The bacterial toxicity testing is still ongoing. Nanoparticle characterisation with UV/vis spectroscopy revealed only a slight agglomeration dynamics independent of soil properties for 3-8Ag, while higher organic matter and low pH appeared to stabilise the 52PVP nanoparticles in suspension. Overall both soil organic matter content as well as pH had a nanoparticle dependent effect on fate as well as toxicity to C. elegans, however, there appears to be no clear connection between the measure particle properties and their effect.

Toxicity and bioaccumulation of Ag NPs in the isopod Porcellioonidus pruinosus

P. Tourinho, University of Aveiro / Department of Biology CESAM; P.V. Silva, Department of Biology CESAM; C. van Gestel, VU University Amsterdam / Ecological Science; A.M. Soares, Universidade de Aveiro / Department of Biology CESAM; S. Loureiro, Universidade de Aveiro / Biology

Silver nanoparticles (Ag NPs) are widely used in nanotechnology industry and consumer products. Due to release from Ag NP-containing products, nanoparticles may enter the soil compartment. Soil properties have a great influence on NP bioavailability and resultant toxicity. It is therefore important to consider the effect of environmental properties on nanoparticle chemistry and form to understand how exposure relevant particles are presented to, and taken up by, organisms. The effect of soil properties on the toxicity of the Ag NPs and ionic silver (as AgNO3) to isopods, using three natural soils. Moreover, to understand Ag bioaccumulation, toxicokinetic tests with Ag NPs and AgNO3 were conducted in one soil. Three soils were used in the toxicity tests, with organic matter content ranging from 1.6 to 13% and pH from 5.5 to 7.8. Specimen, F. et al. 2013. Porcellioonidus pruinosus (>12 mg) were exposed to Ag NPs and AgNO3 in avoidance behaviour and feeding inhibition tests. For the toxicokinetic study, Lufa 2.2 soil was spiked with Ag NPs and AgNO3 at nominal concentrations of 30 and 60 mg Ag/kg. Isopods were exposed to an uptake phase for 21 days, followed by an elimination phase for another 21 days. Uptake (k1) and elimination (k2) rate constants were estimated for one-compartment model, fitted simultaneously to uptake and elimination data. Total Ag concentrations in isopods from the feeding inhibition and toxicokinetic tests were determined by Atomic Absorption Spectrometry (AAS). The isopods were able to detect and avoid Ag at relatively low soil concentrations, but they were more sensitive to AgNO3. AC50 values ranged from 4.2 to 18 mg/kg Ag for Ag NPs, and from 1.4 to 14 mg/kg Ag for AgNO3. Soil properties did not have influence on avoidance behavior. Food consumption was dose-related decreased, with higher EC50s found in the soil with higher organic matter content and pH level. It may suggest that dissolved Ag released from NPs played an important role in determining Ag NP toxicity. Effects on biomass were related to Ag body concentration, but this relationship was not significant for Ag NPs and weak but significant for AgNO3. There was no clear difference in toxicity between Ag NPs and AgNO3. We therefore conclude that ionic Ag poses a greater risk to isopods than Ag NPs. On the other hand, Ag uptake from both Ag NPs and AgNO3 was similar, as shown by the results of the toxicokinetic test. Also, Ag elimination was very slow and did not differ between Ag forms. Isopods have an extremely high Ag accumulation capacity, suggesting the presence of an efficient Ag storage compartment.
susceptible to the accumulation of TiO₂-NPs directly from fertilizer and/or plant protection products or indirectly from the use of wastewater sewage sludge as manure. Moreover, NPs uptake by agronomic cultures and their translocation to edible plants represent a direct way to transfer them into the food chain. Although several studies investigated the accumulation and effects of TiO₂-NPs on different plant species, few were conducted in real farm soil and studied in the soil-crop system. It is known that both TiO₂-NPs and soil OM are ubiquitous for the environment, thus the importance of studying this aspect in real microcosms. Soil organic matter present or added to the soil to improve fertility could be an important factor that influence the mobility and transference of NPs into the food chain. This study shows the preliminary results on the uptake and effects of TiO₂-NPs in crop species growing in agronomic soil with different OM content. Standard germination tests and measurements of seedling growth and root length were performed in matrixes containing different amount of pristine TiO₂-NPs. Moreover, sorghum and pea species grew for 30 days in controlled laboratory conditions in soils with and without organic fertilization and different amount of NPs added (0, 200, 400 and 800 mg/kg soil). In those microcosm growing tests the effect of NPs and OM added to soil were analysed in both plants (Ti accumulation and translocation in plant tissues, biomass, macronutrients content) and soil (bioavailable P and Ti). ICP-OES and UV-Vis techniques were used for the analysis of titanium and phosphorus, respectively. Generally, the germination tests performed in cress showed no significant root inhibition, even though at 800 mg/kg TiO₂-NPs added to the soil without organic fertilization a low inhibition effect was observed respect to the control soil (no TiO₂-NPs added). In the soil with higher OM content the concentration of 200 and 400 mg/kg TiO₂-NPs showed also a slight root inhibition. The roots of both sorghum and pea plantlets showed an increased Ti concentration as a result of increased NPs concentration in soils. A tendency of reduced bioavailable P were found in increased amount of TiO₂-NPs added to soil without OM fertilization after the growth of sorghum plants.

TU263

A floodplain mesocosm system linking fate and effects of engineered nanoparticles at the aquatic-terrestrial interface

Z. Steinmetz, Universität Koblenz-Landau / Environmental and Soil Chemistry; G. Mettavelli, Institute for Environmental Sciences Group of Environmental and Soil Chemistry; H. Vogel, Helmholtz Centre for Environmental Research - UFZ / Department of Soil Physics; M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; W. Manz, German Federal Institute of Hydrology; T. Baumann, Technische Universität München / Institute of Hydrochemistry; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; F. Lanzel, Landesanstalt für Forstwesen und Jagd / Chair of Soil Ecology; S. Klitzke, Federal Environment Agency; F. Seitz, Inst. for Environmental Sciences / Institute for Environmental Sciences; R.R. Rosenfeldt, Universität Koblenz-Landau, Institute for Environmental Sciences / Institute for Environmental Sciences; G.E. Schaumann, University of Koblenz-Landau / Institute of Environmental Sciences

Over the last decades, engineered nanoparticles (ENPs) will undergo chemical and physical transformation processes. The fate, ecotoxicological potential, and mobility of ENPs in environmental compartments will be influenced predominantly by their aging and current speciation status. In order to understand the aging mechanisms and impact of ENP transformations on their distribution and bioavailability in real environmental systems, mesocosm studies can be helpful. A floodplain mesocosm system was developed to assess the fate and effects of ENPs at aquatic-terrestrial interfaces. It is designed for linking aquatic and terrestrial aging of ENPs with their biological effects in one system under near-natural conditions. The floodplain mesocosm enables us to perform long-term (several months) aging experiments with variable environmental conditions including brief dry-downs and re-wettings. The floodplain mesocosm was designed to perform such experiments. The mesocosms were performed in matrixes containing different amount of pristine TiO₂-NPs. Moreover, sorghum and pea species grew for 30 days in controlled laboratory conditions in soils with and without organic fertilization and different amount of NPs added (0, 200, 400 and 800 mg/kg soil). In those microcosm growing tests the effect of NPs and OM added to soil were analysed in both plants (Ti accumulation and translocation in plant tissues, biomass, macronutrients content) and soil (bioavailable P and Ti). ICP-OES and UV-Vis techniques were used for the analysis of titanium and phosphorus, respectively. Generally, the germination tests performed in cress showed no significant root inhibition, even though at 800 mg/kg TiO₂-NPs added to the soil without organic fertilization a low inhibition effect was observed respect to the control soil (no TiO₂-NPs added). In the soil with higher OM content the concentration of 200 and 400 mg/kg TiO₂-NPs showed also a slight root inhibition. The roots of both sorghum and pea plantlets showed an increased Ti concentration as a result of increased NPs concentration in soils. A tendency of reduced bioavailable P were found in increased amount of TiO₂-NPs added to soil without OM fertilization after the growth of sorghum plants.

TU265

Effect of repeated applications of TiO₂ nanoparticles on their transport and toxicity to the microbial community in soil columns

A. Raghavan, Indian Institute of Science, Bangalore / Materials Science and Engineering; M. Simon, Microbial Ecology Lab UMR; G. Uzu, CNRS Université Grenoble 1 / LTHE UMR; J.M. Martins, LTHE University of Grenoble

The soil is exposed to nanoparticles (NPs) due to their use in many commercial products. Chronic exposures lead to an enhancement of NPs concentrations in the soil rising concern about the environmental risk. The adverse effect of NPs on soil microorganisms has been reported in various ecotoxicological studies mainly based on acute tests. The lack of ecological relevance for ecotoxicological studies is a result of the setup of more appropriate systems to study the impact of NPs in conditions closer to the soil exposure mode actually expected. By using soil columns, we assessed the influence of single and repeated applications on the transfer of TiO₂-NPs through soil and we compared the effect of these applications to TiO₂-NPs on the abundance and activity of soil nitrifying microbial community Soil columns consisted in 10 cmheight glass tubes filled with 2 litres of sandy clay loam soil. NPs suspensions were prepared in soil solution and injected in soil columns as a single, two and three exposure conditions with 50, 25 and 16.7 mg L⁻¹ respectively to achieve the same final concentration. The applications were repeated by 15 days of incubation at 28°C. After the last application, all soil columns were cut into layers according to the depth. Each slice was incubated for two months at 28°C. TiO₂-NPs transport was investigated by measuring the Ti content in column leachates. The effect of TiO₂-NPs on the soil microbial community was assessed by measuring the nitrifying activity (NEA) and microbial abundance of nitrifiers (ammonia-oxidizing archaea, AOA, ammonium oxidizing bacteria, AOB and nitrite oxidizing bacteria, NOB) by qPCR targeting functional genes (amoA, nxrA). At the first application, we observed that TiO₂-NPs in soil columns whatever the concentration of the suspension applied. Nevertheless, TiO₂ mobility is higher for the lowest injection concentrations. Interestingly, repeated applications of the same concentration limited TiO₂-NPs transport. The number of exposures impacted the microbial community. Tripletic exposure resulted in a significant decrease of NEA and abundances of AOA, AOB and NOB, whereas no effect was observed for the nitrogenase activity. By different ecotoxicological relevance for ecotoxicological studies, we observed the influence of repeated exposures on NPs transport. Moreover, we highlighted the more detrimental effect of the chronic contamination on the nitrification process than the acute contamination in this soil.
Some answers dealing with emission of pollutants from wastes were given. We used Scanning Electron Microscopy (SEM) to specifically detect the phases bound to metals of colloidal size. Thus, it is of great interest to evaluate the composition of metals under colloidal form present in waste leachates in order to study the phase they are bound to, an essential step for determining the potential environmental impact of ENPs on the environment. This concept follows similar approaches for nutrients and organic contaminants traditionally used in water quality models. Lakes were selected such that lake residence times, depths and areal hydraulic loadings covered the widest possible range among existing lakes. Sedimentation accounted for natural colloidal as well as suspended solid settling regimes for three ENP types: CNTs, ENPs and ENMs. CNTs (through, for example, facilitated nanoparticle transport of organic contaminants, as well as their potential efficiency as a superior sorbent (e.g., for waste treatment water, or for use in solid phase extraction cartridges). CNTs tend to disperse and aggregate in aquatic environments, however, requiring ENPs to be dispersed, which is typically achieved through sonication and surface functionalization. In addition to such intentional modifications, CNT surfaces can also be altered when exposed to oxidative conditions, which is likely to occur during purification treatments and/or after their release into an aquatic environment. The sorption behavior of a series of functionalized CNTs (+OH, -COOH and -NH2) has therefore been systematically evaluated using a passive sampling technique over a wide range of concentrations and dispersion scenarios. Low levels of functionalization significantly affected sorption affinity for pyrene. Sorption greatly increased both the sorption affinity and the maximum capacity of all types of CNTs, to an extent that overwhelmed the differences initially observed (increase of up to 1.5 orders of magnitude lead to log Kd values close to 9 L/kg). Results demonstrated that a significant proportion of the CNT surface was unavailable to pyrene prior to sonication. The presence of humic acids enhanced dispersion but decreased sorption, especially when combined with sonication. Sorption affinity, however, remained very high in all cases (log Kd > 7.5 L/kg), suggesting that CNTs can act as strong sorbents under a wide range of conditions, and may consequently affect the fate of organic contaminants. Kah et al. (2014) Sci. Total Environ. 497–498, 133–8

TU268

Emission of metals under colloidal phase in wastes leachates: ICP-AES and SEM-EDX analysis

a. anderson, University of Toulon / var; S. VILLAIN, University of Toulon; P. HENNEBERT, INERS; P. Merdy, University of Toulon / Chemistry European Institute; G. Lucarini, University of Toulon / Chemistry

In general, CeO2-NPs caused the greatest inhibition in biogas production (nearly 100%) and a strong inhibitory action of other biomasses; Ag-NPs caused an intermediate inhibition in biogas production (33–50%) and a slight inhibition in the action of other biomasses, and Au- and TiO2-NPs caused only slight or no inhibition for all tested biomasses. Also, the maximum toxicological effect that NPs can produce in the biological activity of the microbial communities was studied by calculating the EC50 value. Au-NPs and TiO2-NPs present zero or low toxicity, while Ag-NPs present an intermediate toxicity (inhibition around 33%). Knowledge of the EC50 value for each NP should enable us to anticipate changes in the performance of practical WWTP.

TU269

Sorption behaviour of functionalized carbon nanotubes

M. Kah, University of Vienna / Department of Environmental Geosciences; X. Zhang, University of Vienna; T. Hofmann, University of Vienna / Department of Environmental Geosciences

Understanding the interactions between organic contaminants and carbon nanotubes (CNTs) is essential for evaluating the potential environmental impact of CNTs (through, for example, facilitated nanoparticle-bound cotransport of organic contaminants, as well as their potential efficiency as a superior sorbent (e.g., for waste treatment water, or for use in solid phase extraction cartridges). CNTs tend to disperse and aggregate in aquatic environments, however, requiring ENPs to be dispersed, which is typically achieved through sonication and surface functionalization. In addition to such intentional modifications, CNT surfaces can also be altered when exposed to oxidative conditions, which is likely to occur during purification treatments and/or after their release into an aquatic environment. The sorption behavior of a series of functionalized CNTs (+OH, -COOH and -NH2) has therefore been systematically evaluated using a passive sampling technique over a wide range of concentrations and dispersion scenarios. Low levels of functionalization significantly affected sorption affinity for pyrene. Sorption greatly increased both the sorption affinity and the maximum capacity of all types of CNTs, to an extent that overwhelmed the differences initially observed (increase of up to 1.5 orders of magnitude lead to log Kd values close to 9 L/kg). Results demonstrated that a significant proportion of the CNT surface was unavailable to pyrene prior to sonication. The presence of humic acids enhanced dispersion but decreased sorption, especially when combined with sonication. Sorption affinity, however, remained very high in all cases (log Kd > 7.5 L/kg), suggesting that CNTs can act as strong sorbents under a wide range of conditions, and may consequently affect the fate of organic contaminants. Kah et al. (2014) Sci. Total Environ. 497–498, 133–8
Sorption mechanism of carbonaceous materials for naphthalene, lindane and atrazine: Site energy distribution-based analysis

X. Shen, X. Guo, Peking University / College of urban and environmental science; M. Zhang, X. Wang, Peking University; S. Tao, Peking University / College of Environmental Sciences; B. XING, Department of Plant, Soil and Insect Sciences / University of Massachusetts

Sorption of lindane and atrazine was investigated on ten kinds of carbonaceous materials, including four kinds of graphene, three kinds of graphite, two kinds of carbon nanotubes and one kind of mesoporous carbon, and approximate sorption site energy distributions were calculated based on the Dubinin-Ashtakhov (DA) model. Further more, average sorption site energy and standard deviation of the site energy distribution were deduced and applied to analyze the sorption behaviors of the sorbents, and by this, the site heterogeneity. The introduced oxygen-containing functional groups decreased the average sorption energy of the sorbents for the studied compounds. However, number of sorption sites, indicated by physical properties (i.e. surface area and porosity), still played a more important role in the sorption capacity. Sorption site heterogeneity of the sorbents also decreased as their oxygen contents increased. One reason could be the better dispersion of the oxygen-containing materials as indicated by TEM micrographs. This study put forward a method to quantify the average sorption energy and sorption heterogeneity and made a better understanding of the sorption mechanisms between organic pollutants and carbonaceous materials.  

TU273
Interactive effects of n-TiO2 and 2,3,7,8-TCDD in Mytilus galloprovincialis: a transcriptomic and immunohistochemical study

M. Banni, Laboratory of Biochemical and Environmental Toxology; S. Sforzini, Universita Del Piemonte Orientale Amadeo Avogadro / Department of Sciences and Technological Innovation DiSIT; T. Baihi, University of Genova / DISTAV; A. Giorgi, Universita del Piemonte Orientale / Department of Sciences and Technological Innovation DiSIT; L. Canesi, university of genoa / DISTAV

Exposure of marine organisms to nanoparticles (NPs) in the presence of other pollutants may affect the pattern of contaminant uptake/bioaccumulation as well as toxicity at different levels of biological organization. We have recently shown that in the marine mussel Mytilus galloprovincialis exposure to n-TiO2 is one of the most widespread type of NPs in the aquatic environment in combination with 2,3,7,8-TCDD, chosen as a model persistent organic xenobiotic, can exert antagonistic or synergistic effects on different biomarkers from the molecular to the tissue level, depending on cell/tissue and type of measured response. In order to better understand the interactive effects of n-TiO2 and TCDD in mussel digestive gland, a detailed study was carried out using histology, transcriptomics and immunohistochemistry. To this aim, we employed a cDNA microarray with 1673 sequences to measure relative transcript abundances. In animals exposed only to TiO2, functional genomics analysis of the microarray data (48 differentially expressed genes - DEGs) highlighted three biological processes, largely dominated by the up-regulation of microtubule-based movement-related genes. Exposure to TCDD yielded 49 DEGs exhibiting distinct patterns in terms of biological processes. Finally, exposure to the mixture rendered 62 DEGs that were characterized by the regulation of response to chemical stimulus, microtubule-based movement and intracellular signal transduction. Immunohistochemical evaluation of cytoskeletal components confirmed the effect of the mixture on tubulin expression. Moreover, n-TiO2 reduced TCDD accumulation in the tissue, as indicated by immunofluorescence analysis. Finally, we performed 16S rRNA gene sequencing involving titanium dioxide nanoparticles (nano-TiO2, Aerocido®) on 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD) dependent biotransformation gene expression in juvenile European sea bass (Dicentrarchus labrax). An in vivo 7 days of aqueous exposure was performed with TiO2, TiO2 + 2,3,7,8-TCDD (46 pg/g body weight) and RNA expression of aryl hydrocarbon reector (ahrr), estrogen receptor (er), ABC transport proteins as abcb1, abcc1, cyp1a, p450, cyp2a, gluathione-s-transferase (gst), glutathione reductase (gr) and engulfment and motility (ELMO) domain-containing protein 2 (elmod2) were investigated in liver tissue. Ahrr, er2β, abcc1 and abcc2 were down-regulated with respect to controls in all experimental groups. Co-exposure to nano-TiO2 and TCDD caused a further significant down regulation of ahrr, er2β, abcc1 and abcc2 compared to single chemical exposure (nano-TiO2 or TCDD alone). No effects were observed for TCDD and nano-TiO2 alone in abcc1 gene, while abcc2 was down-regulated by nano-TiO2 alone. Cyp1a, gst and elmod2 genes were up-regulated by TCDD and to a similar extent after co-exposure to nano-TiO2 and TCDD. HR-TEM showed nano-TiO2 (~27nm) which not affected by co-exposure by TCDD. Overall the results indicate that nano-TiO2 is unlikely to interfere with TCDD-dependent biotransformation gene expression in the liver of European sea bass, although additive effects of co-exposure observed in ABC transport mRNAs might suggest effects on xenobiotic metabolite transport in liver.

Adverse outcome pathway concept in research and risk assessment (P)

TU275
Assessment of Adverse Outcome Pathways for Lemna minor in response to single and multiple stressors

L. Xie, Norwegian Institute for Water Research; T. Gomes, Norwegian Institute for Water Research (NIVA); Y. Song, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Management; T. Christensen, Norwegian Radiation Protection Authority; K. Solhaug, Norwegian University of Life Sciences; B. Salbu, Norwegian University of Life Sciences / Department of Plant and Environmental Sciences; K. Tollefsen, NIVA / Ecotoxicology and Risk Assessment

Standard toxicity testing performed in multi-trophic bioassays (e.g. aquatic plants, algae, crustaceans and fish) is applied extensively to predict the effects of chemicals in ecosystems and subsequent risk assessment. Although being highly useful for regulatory purposes focusing on traditional adverse endpoints, these methods provide limited information about the toxic mechanism or mode of action (MoA) of chemicals and rarely address complex environmental issues such as exposure to combinations of stressors (multiple stressors). This applies especially to aquatic plants, which has not received the same attention as algae, crustaceans and fish within scientific and regulatory research. Lemna minor is a freshwater aquatic plant routinely used for assessing chronic toxicity of chemicals using standardized methods. The OECD Growth Inhibition Test (221) makes use of its high sensitivity to toxicants, rapid reproduction capacity, resource-effective exposure format and central role in the aquatic ecosystem. The present study aims to develop an effect toolbox focusing on genomic (e.g. transcriptomics), functional (e.g. Photosystem II inhibition, ROS formation) and adverse (e.g. growth) toxicity endpoints using L. minor as a model organism. Several key stressors will be assessed in combination with nontoxic stressors that are commonly present in nature, including radiation, UV radiation (UVA and UVB) and environmental conditions relevant under climate change scenarios, tested singly and in combination, and their single/combined effects characterized with the suite of methods developed. Successful development of such toolset is envisioned to facilitate linking MoA information to adverse and regulatory-relevant endpoints, potentially through supporting concepts such as Adverse Outcome Pathways (AOP) that may be used to characterize impact of these stressors on the individual and population level. The objectives and results obtained will be presented to illustrate how the present concept may enhance the knowledge about single and multiple environmental stressors in this aquatic plant species.  

TU276
Development of Adverse Outcome Pathways for molting-related developmental effects of emamectin on Daphnia magna

Y. Song, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Management; T. Gomes, Norwegian Institute for Water Research (NIVA); L. Xie, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Management

A number of exogenous compounds have the potential to interfere with the endocrine system of animals and may perturb vital endocrine processes to a degree causing an adverse effect (outcome) on ecologically relevant endpoints such as growth, development and reproduction. These endocrine-disrupting, (ED) effects have been well characterized in aquatic vertebrates and mammals due to a well-defined endocrine system and substantial research effort in the last decades, but knowledge on ED effects in a larger range of species is still poorly characterized. Lack of knowledge on ED effects in invertebrates is currently a major limiting factor to properly perform risk assessment of endocrine disrupting chemicals (EDCs) across species. The present project aims to develop and evaluate adverse outcome pathways (AOPs) for ED in the freshwater crustacean Daphnia magna by linking responses at the molecular level with adverse outcomes (AOs) relevant for regulatory processes. A literature survey was performed first to collect existing knowledge of ED effects on D. magna or closely related crustaceans. Putative AOPs were assembled to identify knowledge gaps prior to experimental validation. Computational and experimental methods were additionally used to identify possible ED targets in D. magna and discover novel EDCs in crustaceans for subsequent verification of potential mode of action (MoA). In vivo exposure studies were then conducted with the endogenous hormone 20-hydroxyecdysone and the insecticide emamectin-benzoate using standardized test protocols and determination of effects on transcriptional, functional and apical toxicity endpoints to identify perturbations in the transcriptional initiating events (MIEs), key events (KEs) and AOs of regulatory relevance.  

TU277
Development of Adverse Outcome Pathways for molting-related developmental effects of emamectin on Daphnia magna
Use of Chemical-Gene and Gene-gene interaction databases to constitute putative AOPs

P. Antczak, University of Liverpool / Institute of Integrative Biology; T. Williams, University of Birmingham / School of Biosciences; F. Falciani, University of Liverpool / Functional Genomics; K. Tollesfson, NIVA / Ecotoxicology and Risk Assessment

Transcriptomics measures alterations in gene activity, by finding differences in the abundance of different messenger RNAs between different biological samples. Traditionally ecotoxicology has assessed biological effect through a number of acute or chronic toxicity endpoints and a small number of endocrine disruptor biomarkers. While these have shown to be informative, they suffer from several limitations. They do not for example allow for identification of potential adverse outcome pathways (AOPs) and they do not provide quantitative assessment of risk nor do they allow prediction of exposure effects. Transcriptomics coupled with a battery of computational modelling tools and the wide-ranging variety of chemical and biological measurements has the potential to link chemical exposure with a physiological outcome. We propose, in the first instance, using a computational biology approach to integrate available transcriptomic information from online databases such as the comparative toxicogenomics database (CTD) and Stitch 4.0 to develop putative adverse outcome pathways. Initially the data is screened for highly confident gene-chemical interactions which allows cross chemical identification of mechanisms of action. In addition, each gene is interrogated for its suitability as a putative molecular initiating event (pMIE). These can include biological targets (e.g. receptors, enzymes and proteins) associated with specific cellular compartments such as membrane, cytosol, mitochondria, the nucleus or even extracellular proteins. By ranking genes on their association with specific cellular compartment we can then define novel pMIEs and putative Key Events (pKE) that are associated with a given adverse outcome and thus form the basis for proposing novel AOPs. Finally by integrating this data with experimentally derived data from high-throughput screening approaches such as Toxcast/Tox21, we can assess the predictive power of additional in silico methods to strengthen the evidence for KE and KE relationships in our putative AOPs. Adverse outcomes can then be assigned via already available and/or expert knowledge, or by assigning chemical-disease associations as are available in the CTD. Acknowledgement - The SOLUTIONS Project is supported by the Seventh Framework Programme (FP7-ENV-2013) of the European Union under grant agreement no. 603437.

TU278 Linking metabolic fingerprints to ecotoxicological effect assessment: the Metabolic Effect Level Index

J. Riedl, Safety in the Food Chain; R. Schreiber, Helmholtz/Zentrum für Environmental Research, Biocatalysis & Analytical Ecotoxicology; M. Ott, TU Bergakademie Freiberg / Analytical Chemistry; H. Heilmeier, TU Bergakademie Freiberg / Working group Biology/Ecology; R. Altenburger, UFZ Centre for Environmental Research / Department of Bioanalytical Ecotoxicology; M. Schmitt-Jansen, UFZ - Helmholtz Ctre Environm. Research / Department of Bioanalytical Ecotoxicology

A major goal of ecotoxicology is the prediction of adverse outcomes of populations from early and sensitive physiological or cellular responses. A snapshot of the physiological state of an organism can be provided by metabolic fingerprints. However, to inform risk assessment multivariate metabolic fingerprints need to be converted to readable endpoints suitable for effect estimation and extrapolation. The responsiveness of metabolic fingerprints to the PS-II inhibitor iproprofuron was investigated using HPLC, amino acid analysis, Hydrophilic and lipophilic leaf extracts were analyzed with GC-MS and preprocessed with XCMS. Metabolic changes were integrated in the quantitative Metabolic Effect Level Index (MELI), allowing effect estimation from Hill-based concentration-response models. Hereby, the most sensitive response was revealed by the hydrophilic MELI, followed by the inhibition of photosynthetic efficiency and one order of magnitude higher, by the lipophilic MELI and shoot length change. 50% of the hydrophilic MELI compares allowing effect estimation from Hill based concentration-response models. These can include biological targets (e.g. receptors, enzymes and proteins) associated with specific cellular compartments such as membrane, cytosol, mitochondria, the nucleus or even extracellular proteins. By ranking genes on their association with specific cellular compartment we can then define novel pMIEs and putative Key Events (pKE) that are associated with a given adverse outcome and thus form the basis for proposing novel AOPs. Finally by integrating this data with experimentally derived data from high-throughput screening approaches such as Toxcast/Tox21, we can assess the predictive power of additional in silico methods to strengthen the evidence for KE and KE relationships in our putative AOPs. Adverse outcomes can then be assigned via already available and/or expert knowledge, or by assigning chemical-disease associations as are available in the CTD. Acknowledgement - The SOLUTIONS Project is supported by the Seventh Framework Programme (FP7-ENV-2013) of the European Union under grant agreement no. 603437.

TU279 The SEURAT-1 ab initio case studies: a proof of concept from theory to application

A. White, Unilever; G. Oueldrao, L OREAL; A. Losia, European Commission DG Joint Research Centre; A. Richarz, Liverpool John Moores University; B. Hardy, Douglas Connect; E. Heinzel, Saarland University; E. Benfenati; F. Bois, INERIS; J. Hengstler, IADB; L. van Gunsvens, Vrije universiteit Brussel; R. Judson, US Environmental Protection Agency / Office of Research and Development; S. Escher, Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM) / Chemical Risk Assessment; T. Gocht, University of Tuebingen / Institute for Experimental and Clinical Pharmacology and Toxicology; C. Mahoney, Procter & Gamble

SEURAT1 is a research initiative aiming at developing knowledge and technology building blocks required for the development of solutions for the replacement of current repeated dose systemic toxicity testing in vivo used for the assessment of human safety. Within the project a proof of concept study integrating the building blocks is currently ongoing to demonstrate the possibility of addressing the safety assessment of chemicals for systemic toxicity using in silico and in vitro tools. The approach is based on the use of putative molecular initiating events (pMIEs) and their putative molecular initiating pathways (pMIP) which allow for identifying the extent of departure for critical decision points and assess this in relation to the applicable exposure scenario for the use case. The quantitative risk assessment or ab initio case study is based on a proof a concept at multiple levels: theoretical, methodological as applied within a toxicological MoA framework. In the scope of SEURAT1, the quantitative assessment of repeated dose toxicity is specifically related to liver hepatoxicity. Several read-across approaches that allow for the assessment of liver toxicity (using putative molecular initiating events (pMIEs)) and the putative molecular initiating pathway (pMIP) were used to determine a relevant biological point of departure for the AOPs and relate this through in vivo to in vitro approaches. The SEURAT1 project is designed to address the uncertainty of adverse risk to consumers under different consumer populations & exposure scenarios and for chronic repeated dose systemic endpoints will also be considered in the ab initio case studies. Work is currently ongoing on three gold compounds: methotrexate, valproic acid and doxorubicin. Preliminary results on these compounds will be discussed.

TU280 Using AOPs to Support Read-Across Predictions - 'testing the hypothesis in Seurat-1'

T. W. Schultz; C. Mahoney, Procter & Gamble; A. Losia, European Commission DG Joint Research Centre; A. Richarz, Liverpool John Moores University; B. Hardy, Douglas Connect; E. Heinzel, Saarland University; E. Benfenati; J. Hengstler, IADB; L. van Gunsvens, Vrije universiteit Brussel; R. Judson, US Environmental Protection Agency / Office of Research and Development; S. Escher, Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM) / Chemical Risk Assessment; T. Gocht, University of Tuebingen / Institute for Experimental and Clinical Pharmacology and Toxicology; E. Berggren, European Commission Joint Research Centre (JRC) / Institute for Health and Consumer Protection IHCP and EU Reference Laboratory for Alternatives to Animal Testing IHC & EU Reference Laboratory for Animal Testing IRL; T.W. Schultz; C. Mahoney, Procter & Gamble

The Read-across approach is driven by the needs of the risk assessor to determine a quantitative point of departure for critical decision points and assess this in relation to the applicable exposure scenario for the use case. The quantitative risk assessment or ab initio case study is based on a proof a concept at multiple levels: theoretical, methodological as applied within a toxicological MoA framework. In the scope of SEURAT1, the quantitative assessment of repeated dose toxicity is specifically related to liver hepatoxicity. Several read-across approaches that allow for the assessment of liver toxicity (using putative molecular initiating events (pMIEs)) and the putative molecular initiating pathway (pMIP) were used to determine a relevant biological point of departure for the AOPs and relate this through in vivo to in vitro approaches. The Seurat-1 project is designed to address the uncertainty of adverse risk to consumers under different consumer populations & exposure scenarios and for chronic repeated dose systemic endpoints will also be considered in the ab initio case studies. Work is currently ongoing on three gold compounds: methotrexate, valproic acid and doxorubicin. Preliminary results on these compounds will be discussed.

TU281 Adverse Outcome Pathway (AOP) of silver nanoparticles in nematode Caenorhabditis elegans

J. Jeong, H. Kim, J. Yang, N. Chatterjee, University of Seoul; H. Eom; J. Choi

SEURAT1 is a research initiative aiming at developing knowledge and technology building blocks required for the development of solutions for the replacement of current repeated dose systemic toxicity testing in vivo used for the assessment of human safety. Within the project a proof of concept study integrating the building blocks is currently ongoing to demonstrate the possibility of addressing the safety assessment of chemicals for systemic toxicity using in silico and in vitro tools. The approach is based on the use of putative molecular initiating events (pMIEs) and their putative molecular initiating pathways (pMIP) which allow for identifying the extent of departure for critical decision points and assess this in relation to the applicable exposure scenario for the use case. The quantitative risk assessment or ab initio case study is based on a proof a concept at multiple levels: theoretical, methodological as applied within a toxicological MoA framework. In the scope of SEURAT1, the quantitative assessment of repeated dose toxicity is specifically related to liver hepatoxicity. Several read-across approaches that allow for the assessment of liver toxicity (using putative molecular initiating events (pMIEs)) and the putative molecular initiating pathway (pMIP) were used to determine a relevant biological point of departure for the AOPs and relate this through in vivo to in vitro approaches. The Seurat-1 project is designed to address the uncertainty of adverse risk to consumers under different consumer populations & exposure scenarios and for chronic repeated dose systemic endpoints will also be considered in the ab initio case studies. Work is currently ongoing on three gold compounds: methotrexate, valproic acid and doxorubicin. Preliminary results on these compounds will be discussed.
School of Environmental Engineering Graduate School of Energy and Environmental system Engineering

Adverse Outcome Pathway (AOP) is a framework to link direct molecular initiating event (MIE) to adverse outcome (AO) at higher level of organization, developed by OECD to increase the confidence of molecular data in risk assessment. With this framework, we can predict apical endpoints (e.g., mortality, reproduction) of the chemical through its effect tests in vitro. Toxicity of silver nanoparticles (AgNPs) has been intensively studied, as they are one of the most widely used and applied nanomaterials. We previously reported AgNPs induced oxidative stress and reproductive toxicity in the nematode Caenorhabditis elegans. In this study, we built AOP of AgNPs in C. elegans. We first built a draft AOP of AgNPs from our previous studies, which constitutes the molecular initiating event, as well as associated adverse outcome, as developmental/endpoint toxicity. DNA damage and oxidative stress responsive signaling pathways seem to constitute as key events between them. To confirm this AOP, we conducted C. elegans developmental test covering whole developmental stage using COPAS Select. Synchronized L1 stage worms were exposed to three different concentrations of AgNPs for 24, 48 and 72h, and their developmental kinetics was investigated by examining exposure time- and concentration-dependent changes of EXT and TOF values. We found AgNPs caused delayed development and at the highest exposure concentration, significant reproduction failure was observed. To experimentally elucidate linkage between MIE and AO, developmental test is on-going using C. elegans functional mutants involved in MIE. Acknowledgement: This work was supported by the grant from the Korea Ministry of Environment as “Environmental Health R&D Program” (201200137009).

TU282

In silico Modeling of Aryl Hydrocarbon Receptor (AhR) Activation M.S. Lawless, J. Ghosh, Simulations Plus Inc; A.C. Lee, Simulations Plus, Inc. / Life Sciences; R.D. Clark, Calscience Environmental & Marine Chemistry Laboratory, TCDD Equivalency Factors to PAHs and assess the risks of PAsHs using cancer slope factors and references doses for TCDD, nor should one use such in vitro results to assign Benzo[a]pyrene (BaP) Relative Potency Factors to other PAsHs. This paper presents a validation comparison of the published TCDD Toxic Equivalency Factors and BaP Relative Potency Factors from various studies, including Machala et al. (2001), Villeneuve et al. (2002), Masunaga et al. (2004), and Pieterse et al. (2004) to published animal carcinogenicity data. There was no correlation between results in AhR binding or enzyme induction assay and carcinogenic potential in animals. USEPA agrees with this conclusion. Windal et al. (2005) stated that PAsHs are “not considered dioxin-like compounds, since they...do not produce AhR-dependent toxic effects.” In addition, EPA (2009) agrees that Ah receptor binding affinity does not correlate with carcinogenicity and cannot be used to derive PAsH Relative Potency Factors: “some PAsHs that strongly activate the AhR, such as benzo[k]fluoranthene (Machala et al., 2001), are only weakly carcinogenic.”

TU284

Chemical characterization and toxicological effects of atmospheric fine particles matter on human bronchial epithelial cells M. Boreje, Z. Dagher, Université Libanaise; F. Ledoux, Université du Littoral Côte d’Opale; A. Verdin, Université du Littoral Côte d’Opale / Unité de Chimie Environnementale et Interactions sur le Vivant UCEIV EA; F. Cazier, Université du Littoral Côte d’Opale; H. Greige, Université Libanaise; P. Shoirai, D. Courcot, Université du Littoral Côte d’Opale.

In October 2013, the International Agency for Research on Cancer (IARC) classified outdoor air pollution and fine particulate matter (PM$_{2.5}$) as carcinogenic to human (Group 1). Despite the clear relationship established by epidemiological studies between PM exposure and the onset of respiratory and cardiovascular diseases, uncertainties remain about the physiopathological mechanisms responsible for these diseases. The aim of this work was to determine the composition of two samples of atmospheric PM$_{2.5}$, collected at urban and rural sites and to evaluate their toxicological effects on human bronchial epithelial cells, BEAS-2B, especially to investigate the metabolic activation of organic compounds, the alteration of epigenetic mechanisms (i.e. microRNAs genes expression), the phosphorylation of H2AX and the telomerase activity. Our results showed a significant increase in CYP1A1, CYP1B1 and AhRR genes expression, miR-21 gene expression, H2AX phosphorylation and telomerase activity in BEAS-2B cells after their exposure to PM$_{2.5}$, both in a dose and site dependent manner. These results showed that PM$_{2.5}$, especially urban PM, are able to induce the expression of metabolizing enzymes which can provide metabolic biotransformation of organic compounds into more toxic and carcinogenic metabolites, and to induce the expression of the oncomiR miR-21 which promotes cell growth and enhances tumor invasion and metastasis in lung cancer. In addition, our results have highlighted the role of PM$_{2.5}$ in the activation of telomerase, which can maintain the telomeres length and subsequently preventing cell death, and have also demonstrated the ability of PM$_{2.5}$ to induce DNA breaks and thus to increase the risk of mutations or chromosomal translocations that lead to genomic instability. All these factors may contribute to cell abnormalities, and thus the development of cancer.

TU285

Toxicokinetic modeling of selected POPs in human breast milk monitoring study J. Vlčáková, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; O. Mikes, Masaryk University; M. Černa, National Institute of Public Health; P. Cugr, Masaryk University, Faculty of Science, RECETOX / RECETOX Research centre for toxic compounds in the environment Persistent organic pollutants (POPs) are known for their harmful effects on the environment, where they can accumulate and pass through food-chains into humans. This study is focused on the risks assessment of the human health outcomes for pregnant women in the Czech Republic (1994-2009; more than 4750 samples) connected to the exposure of POPs. Samples of breast milk were collected and measured (selected POPs: PCBs, HCB, HCHs, DDT, DDE and DDD) in the biobanking of National Institute of Public Health. Hazard quotients for each POP were calculated. The calculations of health risks were carried out using modified PBPK model developed by Trapp et al. The primary route for exposure in PBPK model was assumed to be through the dietary intake. Model was reversed to predict chronic daily doses from the biological data. The final predicted doses from breast milk, in the form of chronic daily intakes (CDI$_{int}$), were compared with the intakes from Czech Dietary Exposure Monitoring (CDEM). The highest risks were estimated for PCBs. Predicted CDI$_{int}$ were generally higher than predicted doses from the food consumption. These facts may indicate new, unknown exposure, which will be discussed.

TU286

Analysis of endpoint-oriented LCA methods assessing impacts of water use

Delving into the planetary boundary concept and issues related to biodiversity and natural resources use in LCA (P)
on ecosystem quality
C. Bouchard, Université Laval; M. Núñez, UR Laboratoire de Biotechnologie de l'Environnement; M. Margni, École Polytechnique de Montréal / Mathematical and Industrial engineering; A. Boulay, CIRAIG - École Polytechnique de Montréal / Chemical engineering department; C. Bulle, CIRAIG - ESG - UQAM / Strategy corporate social responsibility

Assessing biodiversity losses due to water use is making large progress. Many methods addressing a multiplicity of impact pathways dealing both with water consumption, water pollution and water infrastructure exist today. The structure of the characterisation models and indicators of these methods is diverse: some models include a fate sub-model and an effect sub-model whilst others are composed of an indivisible characterisation factor (CF). Some measure damage to aquatic biodiversity whereas some others measure damage to terrestrial biodiversity. Impact units (e.g., PDF, species*year) among models also differ. Given the variety of modelling choices, before ecosystem quality indicators are used all together and combined in a single metric, a detailed analysis of the coherence, complementarity and compatibility of existing characterisation models should be performed. This was the objective of this study. The methods review was based on the analysis of good practice rules for characterisation model development in relation to the following four issues: 1) covered impact pathway (e.g., connection between the inventory flow and the CF); 2) structure of the characterisation model and factor (e.g., one CF vs CMF made of fate and effect factors); 3) fate factor modelling (e.g., good use of steady-state models and data in the modelling of pulse interventions); 4) effect factor modelling (e.g., toxiconomic and geographic coverage). Results show that, in their present form, methods cannot be integrated into a joint indicator. The adoption of guidelines with general recommendations for water use impact assessment modelling may help in enhancing actual problems of incompatibility among existing methods as well as future new developments.

TU287
Land use impact assessment of live swine: A case study of US live swine production comparing three impact assessment methodologies
B. Putman, Biological Engineering; G. Thoma, University of Arkansas / Department of Chemical Engineering; J. Burek, University of Arkansas / Chemical Engineering

Global growth and development place high demand on arable land in an attempt to feed an expanding population that now totals over 7 billion people. The impact of these forces on the capacity of land to provide ecosystem services and support natural assets, like biodiversity, are not well understood. Quantifying the human influence on terrestrial resources is critical to guarantee the longevity of our food systems. The National Pork Board (NPB) commissioned the Center for Agricultural and Environmental Analysis at the University of Arkansas to perform a Life Cycle Assessment (LCA) of United States pork production in order to quantify land use throughout the supply chain. The functional unit is one kg of live swine at the farm gate. The supply chain is divided into two stages: production of swine rations and animal rearing on the swine farm (with three sub-stages for sow, nursery, and finishing barns). This case study applies three preeminent biodiversity impact assessment methodologies on the inventory flows identified by the NPB-LCA. The methodologies chosen were developed, in part, by members of UNEP/SETAC’s Life Cycle Initiative for land use impacts on biodiversity. The methodologies were chosen in order to help the initiative and the LCA community move toward consensus in assessing land use impacts on biodiversity. In this presentation a direct comparison of three of the leading methodologies for assessing land use impacts on biodiversity is made. The study highlights strengths and weaknesses of each approach, as well as the relative difficulty of collect information and carrying out the methodological requirements. The resulting impacts on biodiversity calculated by each impact assessment method are presented graphically to illustrate similarities and differences in results. Preliminary results show that methods requiring species richness are easier to acquire input data. The amount of land occupation is shown to have the greatest impact of biodiversity, which is demonstrated by each of the methodologies. The type of species-area relationship used shows a considerable effect on biodiversity impact.

TU288
Land use biodiversity impact assessment of the Finnish economy
A. Holmg, L. Saikku, Finnish Environment Institute

Land use change as well as land occupation for anthropogenic use are among of the most important threats to biodiversity. Regardless of the importance of the issue, methods for the assessment of land use-related impacts on biodiversity in Life cycle assessment (LCA) are not yet fully established. Biodiversity can be assessed at different scales: spatial, taxonomic, local, regional and global. The indicators should be applicable in the impact assessment of national level economy, which is done with environmentally extended input-output (EEIO) models that combine consumption, production and environmental impacts. A reliable method to link Finnish biodiversity effects to products and services is still lacking. The aim of this study is to explore what are the biodiversity impacts of land-use from the life-cycle perspective in Finland. First, the usability of some of the most prevalent biodiversity indicators will be tested with regular LCA calculations. Second, detailed characterization factors (CF) are developed in particular for Finnish land use and tested at the national level. Indicators used in this study are biodiversity damage potential (BDP) and potentially lost nonendemic species (PLNS), which include the rates of actual extinctions. We study the variation in the results of these indicators with the indicators that take into account the change in the quality of land. To estimate the difference, application of the Heteromyer conset is also applied and the results are compared to the results gained with indicators based on species diversity. Detailed CF’s are developed in particular for Finnish land use and tested at the national level. National boreal data is used to improve the characterization factors. For each existing and new developed biodiversity and characterization factors, we use four products as an example, and use LCA to measure biodiversity impacts directly related to the product, as well as EE-I-O modelling to measure direct and indirect impact related to wood products industries of Finland including impacts abroad. As a result, life cycle indicators that are applicable to be used at a local level, but also to account for impacts abroad, are developed for Finland. The biodiversity impacts of products and services as well as impacts of industries of the Finnish economy can be assessed. With the results we aim to discuss if the indicators measure ecologically relevant issues in terms of ecosystem quality.

TU289
Life Cycle Sustainability Assessment of the Production and Supply of Raw Materials and Primary Energy Carriers: integrating existing frameworks
J. Dewulf, EC JRC - IES / Sustainability Assessment unit; L. Mancini, European Commission Joint Research Centre / Sustainability Assessment Unit; Institute of Environment and Sustainability; G. Blengine, EC-JRC; S. Sala, European Commission DG Joint Research Centre / Institute for Environment and Sustainability; C. Bouchard, Universite Laval; J. Dewulf, European Commission Institute for Environment and Sustainability / Sustainability Assessment Unit; Institute of Environment and Sustainability; D. Pennington, European Commission

The sustainable production and supply of raw materials (‘non-energy raw materials’) and primary energy carriers (‘energy raw materials’) is at stake in many policies: their natural resource base for their production and supply, and the access to are limited; moreover, it is high on environmental and social impact agendas as well. A broad-in scope and quantitative framework that supports decision makers is recommended to make use of raw materials and primary energy carriers more sustainably. The contribution proposes a holistic classification of the full set of raw materials and primary energy carriers our society relies on; this is an essential prerequisite when one aims at developing an Integrated Sustainability Assessment Framework that is to be applicable generically. Overall 85 raw materials and 30 primary energy carriers are identified, grouped respectively into seven and five subgroups. Seeking for comprehensiveness, the proposed framework integrates sustainability issues that have been covered and modeled in quite different frameworks previously: ecosystem services, human life cycle assessment, social life cycle assessment, resource criticality assessment, and particular international concerns (conflict minerals assessment). The resulting four areas of concerns, i.e. environmental, technical, economic and social/societal, have been detailed into 10 specific sustainability concerns. Finally, these concerns have been given shape through 15 indicators enabling the quantitative sustainability assessment of the production and supply of raw materials and primary energy carriers. For the implementation of the framework, expertise and data from quite different fields will be essential: economic, environmental and social impact expertise, including associated models, software and data will be indispensable. Nevertheless, if society and its policy makers strive for sustainability, this challenge is to be taken as it may result in a compass for meeting their objective.

TU290
Life Cycle Analyses of local and imported foods in the United Arab Emirates (UAE)
F.A. AlKadhim, S.L. Knuteson, F. Samara, American University of Sharjah / Biology Chemistry and Environmental Sciences

The UAE relies to a great extent on the import of fruits and vegetables from other countries. Intense pesticide usage, soil and water pollution and industrial emissions raise a question on the food security of these products. Markets for locally grown and organically cultivated fruits and vegetables have been on the rise in the UAE, however, as a desert region, locally grown foods have their own environmental impacts. The paper aims to study the environmental impacts of locally grown and imported foods. On average, heavy metals and organic pollutants in imported crops exceed locally grown crops. Values of certain heavy metals including Lead were higher than the Maximum Daily Intake. As an example, imported cucumber had an average of 0.013 mg/kg of arsenic, but mercury was below the detection limit. Domestic tomatoes and cucumbers had high threshold values but did not exceed the safe level. Similar results were observed for organic compounds. Results on bacterial content of domestic crops and imported crops did not show a significant difference. The emirates of Ras Al Khaimah, Sharjah (Al Dhaib) and Abu Dhabi (Al Ain) have proved to have fertile soil suitable for growing better quality crops; however other areas have poorer arid sandy soils, less suitable for crop production. With minimal

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organic matter in the local soils, fertilizer use increases, leading to possible contamination of groundwater. Many areas in the UAE are dependent on either brackish ground water, leading to salinization of the soils, or desalinated water, with climate change impacts and brine impacts to local aquatic ecosystems. The results of the study show that both imported foods and locally grown foods have large environmental and climate change impacts. A combination of imported and locally grown foods should be used in the UAE, with a focus on advanced sustainable and high yield farming technologies such as hydroponics to produce high quality crops and meet with the demands of a fast growing population.

TU291
The Switch from Coffee to Cocoa: Is it sustainable?
L. Lauren, Easthill; V. Prado, Arizona State University
The United States (US) Department of State is currently funding four projects in Latin America with the goals of modernizing workforce skills, improving trade and competitiveness and fostering sustainable business practices. These projects include an online tool that allows small and medium sized enterprises (SMEs) to calculate energy consumption and GHG emissions and provides tools for continuous improvement in Peru and Chile, a company that provides high tunnels for reduced pesticide use and higher yields of tomatoes and other crops in Guatemala, a consultancy that works within companies on change management and continuous improvement in Mexico, and an education system to improve cocoa yields for small farmers in Nicaragua. For the first time, the department has asked to understand the return on their investment from a sustainability perspective in a way that takes into account the economic, social and environmental opportunities and risks of each stakeholder involved. Sustainability Return on Investment (S-ROI) is being applied to each of these four projects to understand the effect of the US State Department grants and improve their efficacy in the future. S-ROI starts by engaging with the corresponding stakeholders, decision makers and local groups to capture the most significant social, environmental and economic opportunities and risks of the project and to different perspectives. This methodology combines traditional return on investment, environmental life cycle assessment, social impacts and risk assessment via a monetization approach that allows for high uncertainty in estimates. The results show both, the best case and worse case scenario for the return to the investor in monetary terms and the effect on each stakeholder, in and out of the value chain.

TU292
The need for an established allocation method when assessing absolute sustainability on a product level
M. Ryberg, Technical University of Denmark / Quantitative Sustainability Assessment Department of Energy Technology Engineering; M. Oussianiaq; M. Haushild, DTU Management Engineering
Assessment of absolute sustainability within life cycle assessment (LCA) framework is operational on the country scale. However, it is difficult to apply the existing approaches to products, which are typically the scope of LCAs. How should we assess whether a chair is (absolutely) sustainable? If we assess the life cycle of the chair and relate the impact scores to the remaining capacity available for impacts, there is a risk that all products are seen absolutely sustainable. In addition, how should we decide on who can use the remaining capacity? To address these issues there is a risk that all products are seen absolutely sustainable. In addition, how should we decide on who can use the remaining capacity? To address these issues, an allocation method is proposed for dividing the remaining capacity between and within product groups. The method is a two-step method developed based on the annual consumption pattern of an average person in the country and share of products in the group. For example, in the first allocation step, the remaining capacity share allocated to furniture should correspond to the share of an average person’s income that is spent on furniture. In this way the product of the chair is related to the remaining capacity allocated to this particular product group. In the second step, allocation is done between product sub-groups using allocation keys specific to each product group, e.g. mass for furniture, or economic revenue for IT. The proposed method facilitates assessment of absolute sustainability of products within the LCA framework.

TU293
Role of biodiversity in life cycle impact assessment through ecosystem services evaluation: a case study of crop pollination services
B. Othoniel, CRP Henri Tudor / Centre de Recherche Public Henri Tudor (CRP Henri Tudor) / Centre de Recherche des Sciences pour l’Environnement; B. Rugani, Centre de Recherche Public Henri Tudor (CRP Henri Tudor) / Centre de Recherche des Sciences pour l’Environnement CRTE; R. Heijungs, Leiden University / Faculty of Economics and Business Administration Department of Econometrics and Operations Research; U. Leopold, Public Research Centre Henri Tudor / Resource Centre for Environmental Technologies (CRTE); C. Wuthagen, Vrije Universiteit Amsterdam / Faculty of Economics and Business Administration Department of Spatial Economics; C. Braun, Public Research Centre Henri Tudor / Resource Centre for Environmental Technologies CRTE; E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE.
Current life cycle impact assessment (LCIA) methodologies for biodiversity evaluation (i.e. based on species richness and functional diversity indicators) do not account for the role of biodiversity in sustaining human well-being. In effect, while representing the roles of biodiversity in regulating ecosystems’ quality and resilience, they do not consider the benefits to society provided by various biodiversity components. To help fill this gap and thus emphasize the multiple roles of biodiversity in LCA, we present here a complementary approach to current LCIA practices. This is based on the impact characterization of ecosystem services (ES) in relation to the ecosystems’ elements that support their provision. By modelling the supply chain of these services from the multi-functional ecosystems and their composing biodiversity elements to the benefiting society, we are able to retrieve a more comprehensive understanding of the impact assessment of land use changes (used as inventory flows) on biodiversity and ES. To this end, the Multi-scale Integrated Model of Ecosystem Services (MIMES) is used, harmonizing different ecosystems functional models. The impacts of rapeseed production on crop pollination and carbon sequestration services in Luxembourg are assessed to illustrate our approach. After tuning MIMES with data from life cycle inventory and other site-specific parameters, ES flows are characterized in order to assess their monetary value with a marginal pricing approach. As a result, the generated regionalization characterized factors relate land use changes occurring in a certain location to changes in biodiversity composition and ES values in other locations. We obtain three main findings. First, our approach encompasses the joint role of biodiversity within ecosystems to supply different benefits to society. This information, so far lacking in LCA, may be useful to incentivise the conservation and management of biodiversity. Second, the model allows the evaluation of trade-offs and synergies between ES and biodiversity, supporting the identification of multi-functional biodiversity and ES hotspots. Finally, it considers spatially heterogeneous relationships between biodiversity, ecosystems and society. Although not free from shortcomings (modelling improvements are on-going), the proposed approach is a first step of a research project we are pursuing on the implementation of integrated ecological-economic dynamics in LCA of ES.

TU294
Biodiversity characterization factors development for a run-of-river hydropower in Life Cycle Assessment
M. Ryberg, Technical University of Denmark / Quantitative Sustainability Assessment Department of Energy Technology Engineering; M. Oussianiaq; M. Haushild, DTU Management Engineering
Keywords: Biodiversity, run-of-river hydropower, Life Cycle Assessment
Run-of-river (ROR) hydropower runs without water storage and uses the river flow. This kind of plant has several physical impacts on the river. First, a portion of the water could be diverted upstream thanks to weirs in the river channel. Secondly, water upstream the weirs has a lower velocity and induces a deposition of the finest particles. These physical impacts result in a lower biodiversity and change the population of macroinvertebrates and fishes. In this context, the aim is to develop a new characterization factor (CF) for biodiversity in Life Cycle Assessment (LCA) of ROR hydropower. The methodology is based on two databases: one with 473 invertebrates (Tachet’s database) and another one with 26 fishes (curve preference by J. Payet). From these two databases, a new characterization factor (CF) for biodiversity was created with their preference score for five classes of water velocity from 0 (no affinity) to 3 (very strong affinity). The type of river and the geographic affinity are also available in the database. For a diminution of speed class (hypothesis between before and after ROR hydropower installation), if the score is better passing through a lower class, the species is considered non-affected (NA), if the score is worse passing through a lower class other than 0, the species is considered affected (A), otherwise the species is considered disappeared (D). At the end, the percentage of population NA, A and D is known for each speed class diminution. These percentages are also attributed to a flow diminution by converting velocity using the relation: Q (flow in m³/s) = v (speed in m/s) x Sm (Cross section in m²). Percentage are modified to obtain fraction disappeared (PDF) and fraction disappeared + affected (FD) (for one speed class), PDF and FD are integrated over time and space to create biodiversity impacts of a ROR hydropower in PDF/m²/year and PAF/m²/year. Biodiversity CF calculation for a ROR hydropower is ongoing and results will be presented and discussed at the SETAC 2015 Meeting. Taking into account biodiversity impacts is new in LCA. The method based on species traits allows a consideration of the species preferences under physical changes created by ROR hydropower. Implementation of this new biodiversity CF will allow to consider this environmental impact of ROR hydropower in LCA compared to other impacts categories already implemented.

TU295
Hemeroby as an impact category indicator for the integration of land use and biodiversity into the Life Cycle (Impact) Assessment
H. Fehrenbach, IFU-Institute for Energy and Environmental Research; B. Grahl, Fa. Integrail; M. Busch, IFEU Institute for Energy and Environmental Research Traditionally, LCAs carried out by the German Federal Environment Agency (UBA) include the impact category land use based on the metric ‘Degree of naturalness of area’ to determine the land use impacts (Becker et al., 2011). The fundamental idea to characterise ‘naturalness’ as an overarching conservation goal (desired state) forming the basic concept to address selected conservation assets still highly appropriate. The idea central to the concept follows the logic that intact ecosystems are not prone to higher levels of disturbance and negative impacts. Recently the authors have developed the so called hemeroby concept in order to provide an applicable and meaningful impact category indicator for the integration of land use and biodiversity into the Life Cycle (Impact) Assessment. This approach is operationalized by a multi-criteria assessment linking the use of land to different subjects of protection: Structure and functionality of ecosystems, biological diversity and different ecosystem services contributing to human

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wellbeing. In this sense hemeroby is understood as a mid-point indicator giving explicite information on naturalness and providing implicite information, at least partly, on biodiversity (number of species, number of rare or threatened species, diversity of structures), and soil quality (low impact). The hemeroby approach provides a methodology to include the quality of land-use systems focussed on naturalness as conservation goal. Further end-point indicators like biological diversity and ecosystem services are implicitly enclosed. The approach is directly applicable but is currently under development in terms of widening the scope.

TU296 How to link biodiversity evaluation and LCA within the AgBalance evaluation method
P. Salinger, BASF SE / Sustainability Strategy; J. Schoenboehm; C. Koenast, e-syncon; M. Gimpans, BASF SE; M. Frank, BASF SE Agrarzentrum Limburgerhof; A. Ufer, BASF SE / Ecotoxicology
The sustainability evaluation method for agriculture and food supply chains “AgBalanceTM” is based on the principles of life cycle assessments which evaluate impacts which may contain conflicting objectives. For example, a definite farming activity may be positively rated for productivity, but it may be negatively rated for biodiversity development. The intention of AgBalance is to specify alternatives which allow decision makers to prioritize farm management strategies and to specify the respective strengths and weaknesses. Herewith, AgBalance follows the principles of the multi-attribute value theory (MAVT) which allows assessments on the basis of conflicting objectives (v. Witzke 2011). The need to describe quantitatively sustainability (or elements of sustainability which includes biodiversity) has led to develop AgBalance which is based on precursor systems. In building on the environmental impacts and economic costs assessed in the Eco-Efficiency Analysis (Salinger 2005) and the additionally integrated social impact indicators in SEEBALANCE® (Schmidt 2004) (Kölsch 2008), AgBalanceTM was designed as a specific type of a new LCA system to assess the sustainability performance of the production of agricultural goods. Therefore, AgBalance in addition to SEEBALANCE contains a range of new agriculture-specific indicators, namely biodiversity, soil health and land use which were identified and developed in a dialogue with various stakeholders. This holistic system allows assessing parameters which contribute to the development of sustainability in agriculture at several user categories: (1) for the farmers, by assessing current practices and developing scenarios for improved processes; (2) for the agri-food value chain, by assessing agriculture’s contribution over the complete product life cycle and developing options for improvement, and (3) for policy makers, by assessing the impact of legal bodies and regulations on products and farming practices. Depending on the level of assessment of a study, a “balance” between unequally or even controversially directed factors can be evaluated and assessed by the indicator system.

TU297 Ecocentric implementation of the economic dimension into LC + DEA studies
D. Iribarren, Instituto IMDEA Energía / Systems Analysis Unit; M.M. Gamboa, Instituto IMDEA Energía; J. Dufour, Instituto IMDEA Energía / Systems Analysis Unit
Current methodologies for sustainability assessment usually cope with environmental, economic and social aspects of human-dominated activities following an anthropocentric perspective. However, this perspective is insufficient when it comes to assessing the contribution of natural resources to production processes. In order to close the gap, analysing and operationalising the link between economic and ecological systems. This work shows different pathways to implement the economic dimension into the LC + DEA concept, emphasising its capability for taking into account ecosystem services. The LC + DEA concept refers to the combination of life-cycle (LC) approaches with Data Envelopment Analysis (DEA) for the sustainability assessment and benchmarking of multiple similar entities. In particular, the Em + DEA method has the potential to translate emery benchmarks into market-driven indicators for the valuation of ecosystem services. Solutions to enhance the economic dimension of LC + DEA studies have recently been proposed in order to deal with environmental, economic and social aspects in balance. Two different perspectives are distinguished. On the one hand, the traditional anthropocentric perspective focusses on the estimation of the economic savings directly linked to the calculated operational benchmarks. On the other hand, the novel ecocentric perspective quantifies external cost savings as well as emergy savings, relying on the previous calculation of environmental and energy benchmarks. The application of this methodological framework is illustrated with a case study of 25 wind farms located in Spain. Overall, significant direct economic savings and emergy savings are found for the inefficient wind farms, while low external cost savings are found due to the good environmental performance of wind power in terms of air pollution, yet it is concluded that the economic dimension of LC + DEA studies can be feasibly enhanced by calculating not only direct economic savings linked to operational benchmarks, but also external cost and emergy savings linked to environmental and energy benchmarks. This sense, anthropocentric and ecocentric perspectives are found to complement one another, providing decision makers with a valuable set of economic indicators. This enhanced LC + DEA methodological framework enriches sustainability assessment and strengthens the analysis of the interactions between ecosystem elements and human-driven processes.

TU298 Assessing sand and gravel use in LCIA: state of the art and perspectives
E. Loiseau, UMR ITAP, P. Loubet, Veolia Eau dille-de-France / UMR ITAP ELSA; A. Helias, INRA; P.C. Roux, Iresta / UMR ITAP ELSA
Sand and gravel are the third most consumed resource in the world after air and water. These resources are required for infrastructure and building, and for other purposes such as agriculture, which are sometimes almost unlimited at a global scale. Nonetheless, human users may face local or regional depletions, and other sources of sand and gravel are thus being sought. Among them, marine aggregate extraction is getting more and more important. This intensive extraction of resources could have significant impacts on the environment, sometimes resulting in harmful socio-economic and political effects. The most striking example is the disappearance of sand islands in Indonesia. In light of these findings, it seems paramount to account for the impacts of sand and gravel extraction in Life Cycle Impact Assessment (LCIA). However, little attention was paid on these issues in LCIA methods. This paper aims at establishing a preliminary LCIA framework to address sand and gravel use impacts in a comprehensive way. To this end, a review was performed on the effects of sand and gravel extraction both as (i) a resource used for different human activities (e.g. construction) leading to abiotic depletions and (ii) as a contributor to impacts on different land categories (e.g. mineral extraction sites, coastal regions or seabeds). The different LCIA methods were assessed in order to determine how sand and gravel are currently taken into account. Only certain LCIA methods account for sand and gravel depletion at a global scale. In addition, except for land occupation assessment, LCIA methods are mainly a tool for the calculation of direct external costs due to sand and gravel extractions on natural ecosystems (e.g. coastal land or water bodies). Yet, many environmental mechanisms have been inventoried such as land destruction, erosion, seafloor destruction, inland salt water intrusion or water table lowering. From the existing frameworks designed for assessing the impacts of land use, water use and sea use in LCIA methods, we identified the possible impact pathways linking sand and gravel extractions to impact categories already used in midpoint and endpoint levels. Moreover, we emphasized the fact that human users can potentially develop compensations scenarios to adapt to a resource depravation and that the indirect impacts due to these scenarios should also be quantified. The resulting preliminary framework paves the way for the future developments required to quantify all the impacts of sand and gravel extraction.

TU299 Towards operationalizing the Planetary Boundaries concept in LCA for products
R. Murphy, R. Clift, University of Surrey / Centre for Environmental Strategy; H. King, Unitled; S. Sim, Unilever; R.D. Colwell, Safety and Environmental Assurance Centre SEAC; J. Chenoweth, I. Christie, University of Surrey / Centre for Environmental Strategy; J. Clavreul, Univerley R&D Colwell / SEAC; J. Lee, University of Surrey / Centre for Environmental Strategy; R. Clift, University of Surrey
The Planetary Boundaries (PB) concept of Rockström et al (2009) has attracted significant international interest and an approach has been defined as an ‘absorbing the impacts of sustainability’ space within which mankind needs to remain. This recognition of a safe operating space has clear relevance to LCA work and is potentially a novel perspective on a ‘distance to target’ approach. We have been working with a group of international experts to explore the science underpinning the PBs approach and to explore aspects of operationalising the PB concept in the fast moving consumer goods sector. Traditional LCA approaches are already used in the sector and we are currently researching the potential for adaptation of LCA methods as a potential tool to enable operationalization of absolute sustainability metrics based around PBs. In this presentation we examine the opportunities and limitations for operationalising the PBs concept in LCA with particular regard to the Biodiversity, Chemicals and Water boundaries.

TU300 Towards a common understanding of the Area of Protection 'Natural Resources' in Life Cycle Assessment
J. Dewulf, EC JRC - IES / Sustainability Assessment unit; L. Benini, JRC Institute for Environment and Sustainability Assessment Unit; M. Crecioni, EC JRC - IES; J. Maes, EC-JRC; R. Pant, European Commission DG Joint Research Centre / Institute for Environment and Sustainability Sustainability Assessment Unit; G. Blengini, European Commission DG Joint Research Centre; F. Ardente, Joint Research Centre / Sustainability Assessment Unit; M. Recchi, European Commission JRC Joint Research Centre / Institute for Environment and Sustainability Sustainability Assessment Unit; P. Dennington, European Commission Life Cycle Impact Assessment (LCIA) in classical Life Cycle Assessment (LCA) aims at analyzing potential impacts of products and services typically on three so-called Areas of Protection (Aop): natural environment, human health and natural resources. This contribution proposes an elaboration of the Aop Natural Resources. It starts with analyzing different perspectives on Natural Resources as they are somehow sandwiched in between the Natural Environment (their cradle) and the human-industrial environment (their application). Reflecting different
viewpoints, five perspectives are developed with the suggestion to select three in function of classical LCA. They result in three safeguard objects: the Asset of Natural Resources, the Provisioning Capacity of Natural Resources, and the Global Functions of Natural Resources. Whereas the Provisioning capacity is fully in function of human, the global functions go beyond provisioning as they include non-provisioning functions for humans and regulating and maintenance services for the globe as a whole, following the ecosystem services framework. A fourth and fifth safeguard object is identified: recognizing the role Natural Resources for human welfare, either specifically in their role in supply of products and services and also more in general. But as these are far broader as they in principle should include characterization of mechanisms within the human industrial society, they are considered as objects for an integrated sustainability assessment (LCSA: Life Cycle Sustainability Assessment), i.e. incorporating social, economic and environmental issues.

Midpoint or single score for decision making? (P)

TU301 Screening of sustainability indicators for bioenergy systems: Are they suitable for single-score aggregation?

M. Martín-Gamboa, Instituto IMDEA Energia / eS.LAC Network Systems Analysis Unit; D. Irigarren, Instituto IMDEA Energía / Systems Analysis Unit; M. Schröter, Karlsruhe Institute of Technology / Institute for Technology Assessment and Systems Analysis ITAS; D. Garraín, Ciemat / Energy Systems Analysis Unit; Y. Lechon, Ciemat / Energy Dpt Energy Systems Analysis Unit; W.R. Poganziet, Karlsruhe Institute of Technology / Institute for Technology Assessment and Systems Analysis ITAS; J. Dufour, Instituto IMDEA Energía / Systems Analysis Unit Governments and decision makers are increasingly aware of the need for assessing sustainability, which is especially relevant to the energy sector. In this respect, Life Cycle Sustainability Assessment is considered to be a promising methodology for the evaluation of energy systems. However, a careful identification of performance indicators is required. Within the framework of the EERA JP e3s (www.eera-jp-e3s.eu), a review of more than 100 articles and reports on the evaluation of energy systems is carried out. In order to facilitate the identification of appropriate sustainability indicators, an analysis at the sub-sector level is proposed. In particular, this study shows a thorough selection of sustainability indicators for bioenergy systems. The recommendation of indicators for the sustainability assessment of bioenergy systems is based on the screening of more than 600 indicators reported in the literature. A pre-screening of indicators reduces this number to approximately 150 by removing recurrent indicators. A final screening results in less than 30 indicators to provide a complete picture of the sustainability performance of bioenergy systems. This final screening is carried out according to a set of defined criteria including: life-cycle perspective, practicality (linked to availability and reliability) and specificity for the assessed sub-sector (relevance). The set of recommended indicators is divided into the three common sustainability dimensions (environmental, economic and social) while also including multi-dimensional indicators such as cumulative energy demand (technical and environmental dimensions), external cost (economic and environmental dimensions) and labour (socio-economic indicator). Both general (e.g., global warming, eutrophication, net present value and job creation) and bioenergy-specific (e.g., food, water and agricultural security) indicators are included in this selected set. The suitability of the selected indicators for aggregation when it comes to further interpreting and reporting the sustainability of bioenergy systems is discussed. Two levels of aggregation are explored: intra-dimensional aggregation (one single score per sustainability dimension) and multi-dimensional aggregation (one single score for sustainability). Overall, despite the ease with which results are reported using a single score, aggregation is not seen as a robust approach to support sensible decision making within the framework of sustainability goals.

TU302 Midpoint vs single score in multi-criteria optimization under LCA constraints: the case of potable water treatment chains

F. Capitanescu, CRTE; E. Igoa, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE; A. Marvuglia, Luxembourg Institute of Science and Technology (LIST) / Resource Centre for Environmental Technologies CRTEResource Centre for Environmental Technologies CRTE; E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE In order to offer more efficient and reducing the environmental impact of a process, Life Cycle Assessment (LCA) needs to be complemented by multi-goal optimization tools. The paper reports the successful coupling between a multi-goal optimization tool and EVALEAU, which is a state-of-the-art flowsheet-based process modelling - LCA tool for prospective and retrospective simulation of potable water treatment chains. The EVALEAU simulator was developed in the United Kingdom, relies on the software PHREEQC® for water chemistry calculation, and its modules are linked to the Ecoinvent® database for the LCI of background processes. Since EVALEAU simulator involves a such deep level of software embedding the use of mature derivative-based mathematical programming methods is unreasonable. Consequently, key design operational parameters of the potable water plant treatment chain were optimized by resorting to a metaheuristic global optimization algorithm, called Strength Pareto Evolutionary Algorithm (SPEA2), which is a natural candidate for black-box multi-objective optimization. The work also assesses the pros and cons of using midpoint versus single score as environmental goal metrics in a decision making process based on very challenging multi-goal optimization of water treatment chains. The problem computational challenges stem from the facts that EVALEAU is a time consuming simulator and the computational complexity (i.e. approximation of Pareto front and running time) of such multi-objective optimization problems is extremely high due to the presence of conflicting objectives and may be unmanageable. The results show that solving such optimization problems under both approaches is technically feasible provided that, for midpoint approach, a small number of key conflicting environmental impact metrics are carefully chosen by the decision maker. Other midpoint impact categories can be straightforwardly incorporated into the optimization problem as constraints but the optimization results may depend on their apriori expert-based limits.

TU303 LCA in multicriteria optimization tools for process design: sewage sludge-based biodiesel production

C.M. Torres, Departamento de Ingeniería Química; M. Olkievich, Universitat Tovira i Virgili / Chemical engineering department; F. Castells, Universitat Rovira i Virgili / Chemical engineering department; C. Bengoa, L. Jiménez, Universitat Rovira i Virgili / Chemical Engineering Decision-making tools for process design must combine both economic feasibility and preferable impact evaluation in order to meet the stakeholders’ interests. It is the aim of the ongoing researches in alternative raw materials and processing technologies to produce biodiesel. This study analyses from a realistic point of view the biodiesel production from primary sewage sludge by a simulation-based automated evaluation tool. This computational procedure allows automating the calculation of both mid-point and aggregated multicriteria indicators, the checking of the key parameters, the assessment of hot spots through sensitivity analysis, and the implementation of uncertainty analysis under several scenarios and process assumptions. The automated characterization approach is based on the plant simulation in AspenHysys v8 and the automated iterative process programmed in Matlab to establish the pattern given by the optimization algorithm, uncertainty model or sensitivity analysis procedure to be followed. Three process alternatives were selected to find the best technological solution: a novel wet extraction procedure (Wet route alternative) developed at laboratory scale versus traditionally alternatives from dry sludge, using conventional dry extraction with hexane (DryA alternative) and in situ transesterification route (DryB alternative). The alternatives comparison in the base case scenario is complemented by a sensitivity analysis to check the importance of the assumptions made during the inventory phase. Additionally, Monte Carlo analysis was performed over the correspondent distribution functions defined for the key operational variables, the predefined economic parameters and the characterization factors of the LCA. The resulting simulation gives no significant difference between the LCA of the Wet and DryA routes, while the better economic results points out the Wet alternative as the preferable option with 98% of confidence. The available computational tools allow us to better estimate, analyse and draw conclusions about the environmental evaluation through LCA, taking into account multiple scenarios, assumptions and design choices, without having to assume certain parameters and keeping a detailed perspective in each step of the analysis. The use of appropriate techniques can give a broader view of the process performance avoiding to face tiring calculations and a vast number of scores.
the average carbon footprint of banana farming was reduced by 15% when applying these optimization measures. The application of the CF+DEA method was found to be a valuable tool for farming optimization, allowing an easy estimation of the environmental improvements that would result of the application of given target values to inefficient units. DEA constitutes, however, a complex model, leading to the conclusion that results should be carefully analyzed to ensure target viability before implementing any optimization measure.

TU305 Environmental footprint of the company in consideration of the whole supply chain
S. Suguri, N. Isukub, Tokyo City University
In late years, the attention for the environmental evaluation of the organizations in the LCA. For example, I include "Organization Environmental Footprint" which introduction is considered for in Europe, "PUMA's Environmental Profit and Loss Account for the year ended 31 December" by PUMA company. In this study, I performed examination for the development of the environmental footprint technique for the company for the whole supply chain. For end-point, I aim at covering all influence domains associating with Organization Environmental Footprint in Europe I evaluate it using a Japanese Input-Output table analysis in this study, and the input data used the disclosure data of the company.

TU306 Eco-efficiency indicators for decision-making support in the Urban Water Sector
D. Marin, M. Amores, CETAqua, Water Technology Centre; Y. Lorenzo-Toja, USC; D. Sanjuan-Delmas, UAB; A. Petit-Boix, Institute of Environmental Science and Technology; C. Arnal, CETAqua Water Technology Centre; G. Feijoo, University of Santiago de Compostela / Chemical Engineering; J. Rieradevall, Institute of Environmental Science and Technology ICTA Universitat Autònoma de Barcelona / sostenipena; M. Termes-Rifé, Universitat de Barcelona / Department of Economic Policy Faculty of Economics and Business; F. Hernandez-Sancho, University of Valencia
The recently released standard for eco-efficiency assessment (ISO 14045:2012) is proposed to support decision-making when dealing with urban water cycle management. The lack of such an integrated assessment may lead to non-eco-efficient performances both from the economic and the environmental point of view. The most appropriate environmental, economic and eco-efficiency indicators were selected after the application of both LCA and LCC to the urban water cycle activities of two small-medium cities in Spain. Nevertheless, with respect to LCA results, midpoint and endpoint indicators have been compared in order to select which were the most understandable ones for end-users. The midpoint indicators selected were Global Warming (GWP), Ozone Layer Depletion (ODP), Eutrophication (EU) and Cumulative Energy Demand (CED) as those were the common relevant indicators in the four stages of urban water cycle (drinking water treatment, supply network, sewer network and wastewater treatment and discharge). Both construction and operation and maintenance impacts were included as for treatment plants the most important stage is Operation and for networks it is more relevant the construction stage. Regarding the economic assessment, besides LCC, cost functions were developed in order to estimate costs for water facility and monetisation of externalities was also carried out in order to have a single economic indicator including, costs, monetised environmental impacts and monetised environmental benefits that can be understood by itself. With respect to the eco-efficiency assessment, the previous works from academia and companies showed that there are many different ways to assess the “value” of a product or activity while the standard clearly identifies LCA results as the most appropriate indicators for the environmental assessment. In the case of urban water activities, LCA midpoint and endpoint indicators have been used to correlate them with economic indicators (CAPEX, OPEX and externalities) and also with quality indicators (e.g. BOD reduction or GHG emissions). The most preferred results were the ones that have a graphical interpretation that best supports decision-making. A user friendly web tool for eco-efficiency assessment in the urban water cycle devoted to non-life-cycle experts has been developed gathering all this knowledge and as a collaborative work within academics, water utilities and public institutions.

TU307 Carbon footprint of plant derived surfactant use in leather industry
E. Kilic, E.Scola d'Enginyeria d'aguadalu / Textile and Leather Fashion Design Department; R. Puig, A. Navarro, Universitat Politècnica de Catalunya / GIR Escola d'Enginyeria d'aguadalu / Textile/Leather Fashion Design
In leather production degreasing is an important processing step where the excess of natural fat substances are removed from animal skin. The conventional degreasing methods used for degreasing of skins generally involve using polluting products such as organic solvents and/or synthetic surfactants, or their mixture in the form of aqueous emulsions. The industrial application of these methods has been questioned and raised the question of sustainability because of non-biodegradable structure of synthetic chemicals. Surfactants are shown to be toxic to aquatic organism and their presence generate severe environmental burdens due to use of large amounts of water and cause significant problems in the conventional wastewater treatment processes. The possibility of using plant derived biosurfactant in the degreasing of sheep skins have been investigated and biosurfactant based degreasing process has been described as a viable and promising ecological degreasing option for leather industry. This study analyzes the environmental performance of the biosurfactant based degreasing process in leather industry in comparison to conventional process where synthetic surfactants are used. Life Cycle Assessment (LCA) methodology is applied and biosurfactant use was evaluated by analyzing the global warming potential impact category defined by CML 2001 (measured in kg of CO2 equivalent emissions), water and energy consumptions.

TU308 Benchmarking carbon footprint of wine industry: comparing data from 20 wineries in Spain and France
A. N. Diante, R. Puig i Vidal, Universitat Politècnica de Catalunya / GIR Escola d'Enginyeria d'aguadalu; E. Kilic, LEscola d'Enginyeria d'aguadalu / Textile and Leather Fashion Design Department; P. Fullana i Palmer, Universitat Pompeu Fabra / UNESCO Chair in Life Cycle and Climate Change
Inventory data were obtained from 20 wineries with varying production volumes participating in two projects coordinated by Cyclus: 8 within the CO2 Vino project (financed by the European Social Fund through the Program empleaverde from Diversity Foundation) and 12 within the VINECO project (financed by the Euroregion Pyrenees-Mediterranean). The wineries were distributed in 12 designations of origin from 5 regions in Spain (Galicia, Castilla La Mancha, Murcia, Catalonia and Balearic Islands) and 2 in France (Mid Pyrenees and Langedoc-Rousillon). Both projects had as a goal the fight against climate change, together with increasing competitiveness by delivering environmental information to sensitized markets. In addition to CO2 emissions, the VINECO project aimed at evaluating and reducing water and energy consumption as well. A comparative analysis of the inventory data collected to calculate the carbon footprint of the 20 mentioned wineries is presented. Specifically, a comparative statistical study of consumptions of chemicals and energy per hectare of vineyard, per kg of grapes and per liter of wine produced has been performed. Different variables have been identified as relevant to the results, such as fuel consumption, use of phytosanitary products and weight and type of packaging. The comparative results obtained in this study can be used by wineries as a benchmark to compare their impacts to the average and identify what variables are causing their deviation from the average. This may help and encourage wineries to adopt measures to reduce their carbon footprint.

Assessment of risks posed by systemic insecticides to hymenopteran pollinators: from lab via (semi-)field to landscape scale testing (P)

TU309 Spatial representativeness of field studies on neonicotinoids used for the EU risk assessment to bees
A. Ippolito, EFSA - European Food Safety Authority / Institute for Environment and Sustainability IES; M. Arena, EFSA - European Food Safety Authority / Pesticides; D. Auteri, Auteri / Pesticides Unit; T. Molnar, R. Sharp, EFSA - European Food Safety Authority / Pesticides Unit; F. Streissl, EFSA / Pesticides Unit; A. S. Szentess, Pesticides Unit

In view of the various studies and research activities carried out in recent years, in 2012 the EU Commission requested EFSA to revise the risk to bees of neonicotinoid active substances by focussing on clothianidin, imidacloprid, and thiamethoxam applied as seed treatments and granules. In particular EFSA was tasked to consider the acute and chronic effects on colony survival and development, effects on forage and bee behaviour, including effects of sublethal doses. The mandate recommended to perform the risk assessment on the basis of the EFSA PR Panel Opinion, 2012. The risk assessment for honey bees concentrated on three main routes of exposure: via intake of residues in nectar and pollen collected in the flowers of treated plants; from dust produced during the sowing or the application of the granules and drifting on to nearby vegetation; via intake of residues in guttation fluid produced by the treated plants. To this purpose, a number of semi-field and field studies were submitted for the approval of the active substance at EU level and in support of the product authorisations at Member State level. These studies were conducted under certain environmental conditions (e.g. specific crop, landscape, and climate) and therefore their representativeness for different regions and environmental conditions in Europe was questionable. In the present work, the aforementioned studies will be geographically characterised using maps, their spatial relationship with the distribution of bee-attractive crops and biogeographic regions across Europe will be shown. Uncertainties in extrapolating results from field studies to a pan-European scale will also be discussed. To enhance the interpretation of the results, a short meta-analysis will briefly highlight the main issues undermining the reliability of some studies.

TU310 Calculators developed for the EFSA Guidance Document on Risk Assessment of Plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)
C. Szentess, Pesticides Unit; G. Zancanaro, European Food Safety Authority / AMU; F. Streissl, EFSA / Pesticides Unit

310 SETAC Europe 25th Annual Meeting Abstract Book
New perspectives on the risk assessment of PPPs to bees were provided by EFSA’s new guidance document. This guidance suggests the implementation of tiered risk assessment schemes (screening, first tier, second tier, highest tier). The guidance document was heavily criticised by different stakeholders as being too complex at lower tiers and difficult to apply refined risk assessments at higher tiers. The high number of risk quotients, which need to be calculated for the lower tiers, originates from the complexity of the different exposure routes in agricultural landscapes. The complexity is due to different combinations of default factors for the exposure estimations of the different situations. In order to ease the required calculations, a user-friendly calculator based on MS-Excel named ‘Bee-Tool’ had been developed by a small working group of EFSA. The Bee-Tool is able to quickly perform all the necessary calculations for the screening step and for the first tier. This tool can be used for all risk assessment procedures as most complex factors used in the exposure estimation are the ‘Shortcut values’. The default shortcut values (90th percentile ‘worst case’) were derived by Monte-Carlo simulations considering a set of default residue levels in pollen and nectar and information on feed consumption of bees. Among others, these shortcut values can be refined at second tier of the risk assessments if compound or crop specific data are available (i.e., residue levels in pollen and nectar of the pesticide under evaluation, crop specific sugar content of the nectar, crop specific pollen consumption). In order to ease the calculations of crop or compound specific shortcut values, the so called ‘SHVAL’ tools were developed by EFSA. SHVAL tools are small applications developed for ‘R’ statistical software. The SHVAL tools allow for inputting raw data sets as well as reference values (central tendency measurements / ranges). First, where possible, they try to be based on distributed bees in the sprayed field and then run a Monte-Carlo simulation. The simulations result in an estimate of the 90th percentile shortcut value and its 95% confidence interval. Graphical presentations of the fitted distributions and for the simulated shortcut values are also available. The presentation will give an overview of these calculator tools. Also, with some examples, it will be demonstrated how these calculators work in practice.

TU311

Dilution at the hive - a Dutch field study

I. Roessink, Alterra; D. Belgers, Alterra Wageningen UR; J. Van der Steen, Wageningen UR; A. Focks, Alterra Wageningen University and Research Centre / Ecotoxicology Environmental Risk Assessment Team; h. baveco, Environmental Risk Assessment Team; J. Boesten, Alterra / ERA team.

This study was performed to assess potential dilution of pesticide concentrations entering the bee hive. For this purpose two flowering fields of oilseed rape (Brassica napus) (A and B), situated 650m apart, were sprayed with a separate tracer each. Tracers comprised bosalid and metconazole, both non-toxic for bees. After application, the bees foraged simultaneously in the sprayed field and at the entrance of the beehive. In addition, foraging bumblebees were collected in the field as well. On the bosalid treated oilseed rape field, concentrations in nectar collected from field-bees approximated 300 ng/g immediately after application, resulting in an Residues per Unit Dose (RUD value) of about 3 mg/kg. However, concentrations in nectar collected from returning bees in front of the hive situated neay to the treated field were much lower, data were not available. Interestingly, no metconazole was measured in these samples as well. This indicates that even with an attractive crop such as oilseed rape at close distance, bees may still use a significant other part of the landscape to forage, leading to dilution of initial nectar concentrations as observed in the field.

TU312

Appropriate use of available evidence - an example of exposure of bees to in-field weeds

S.K. Maynard, Syngenta / Environmental Safety; P.C. Campbell, Syngenta / INRS-ETE

In recent years the European Food Safety Authority (EFSA) has increased its programme of preparing guidance and opinions in the field of environmental risk assessment. Many of the current and proposed risk assessment scenarios are worst case and are based on theoretical assumptions that certain scenarios could constitute a risk rather than based on evidence of actual risk. In July 2013 the European Food Safety Authority (EFSA) released its final guidance on the risk assessment of plant protection products (PPPs) to bees (EFSA, 2013). This scheme requires a first tier assessment through the use of a scenario which is a worst case exposure of bees through foraging on attractive weeds within the treated field. The guidance suggests, as a refinement option, that if < 10% of the area of use is covered in attractive weeds then the exposure route is not relevant in the 90th/ile case. Efficacy trials for herbicides follow common practices throughout Europe, are different in their measurement coverage in-field, and are of a standard suitable for regulatory submissions. This project aims to, using this empirical evidence, answer the question posed by the EFSA bee guidance document regarding the relevance of the weeds in the treated field scenario: “Is a significant fraction of the surface area of treated fields covered by attractive weeds for >10% of the area of use?” Using a database of around 11500 efficacy trials (containing approximately 67000 individual weed recordings) across 9 separate crop groups data was extracted on weed species, growth stage and ground cover. Initial analyses demonstrate that of all weeds recorded < 2% were at a flowering growth stage. For permanent crops this value was higher (36%) due to differences in agricultural practices. However, future analyses considering the attractiveness of flowering weeds to bees and the actual ground coverage of weeds is considered these values will significantly reduce these figures and more robustly quantify the exposure to weeds. This large data analysis has demonstrated that the scenario of weeds in the treated field is not applicable for the crops assessed here (wheat, oilseed rape, sugar beet, sunflower, potatoes, maize, peas and permanent crops (orchards and vines)). This work also demonstrates that the currently available regulatory data submitted to member states and EFSA during submissions could be used more effectively when preparing opinions and guidance documents.

TU313

A pragmatic approach to assess the exposure of the honey bee (Apis mellifera) when subjected to pesticide spray

Y. Pagnoulle, D. Giraud, INRA; L. Boesten, Alterra; M. Tchiamaithinchin, INRA; M. Fussellier, National Veterinary School (Oniris); B. Giroud, Institut des Sciences Analytiques UMR TRACES Team; F. Lafay, CNRS Université Lyon; A. Bulete, Institut des Sciences Analytiques UMR TRACES Team / Service Central d’Analyse; S. Tchamitchinchin, M. Cousin, M. Pelissier, J. Brunet, INRA; L. Belzunceas, INRA / Univ Abelles Environment

Plant protective spray treatments may expose non-target organisms to pesticides. In the pesticide registration procedure, the honey bee represents one of the non-target model species for which the risk posed by pesticides must be assessed on the basis of the hazard quotient (HQ). The HQ is defined as the ratio between environmental exposure and toxicity. For the honey bee, the HQ calculation is not consistent because it corresponds to the ratio between the pesticide field rate (in mass of pesticide/ha) and LD50 (in mass of pesticide/bee). Thus, in contrast to all other species, the HQ can only be interpreted empirically because it corresponds to a number of bees/ha. This type of HQ calculation is due to the difficulty in transforming pesticide field rates into doses to which bees are exposed. In this study, we used a pragmatic approach to determine the apparent exposure surface area of honey bees submitted to pesticide treatments by spraying with a Potter-type spray, the doses received by the bees were quantified by very efficient chemical analyses, which enabled us to determine an apparent surface area of 1.05 cm²/bee. The apparent exposure area was used to calculate the exposure levels of bees submitted to pesticide sprays and then to revisit the HQ ratios with a calculation mode similar to that used for all other living species. X-tomography was used to assess the physical surface area of a bee, which was 3.27 cm²/bee, and showed that the apparent exposure surface was not overestimated. The control experiments showed that the toxicity induced by doses calculated with the exposure surface area was similar to that induced by treatments according to the European testing procedure. This new approach to measure risk is more accurate and could become a tool to aid the decision-making process in the risk assessment of pesticides.

TU314

Assessing the potential effects and risk of chronic thiamethoxam exposure to honey bees: results of a hive feeding study conducted in North Carolina, USA

J. Omerver, Syngenta Crop Protection, LLC. / Environmental Safety; F. Rice, Syngenta Crop Protection LLC; S. Bocksch, Eurofins Agroscience Services GmbH; H. Hill, Eurofins; W. Hightower, North Carolina State University

In recent years the European Food Safety Authority (EFSA) has increased its programme of preparing guidance and opinions in the field of environmental risk assessment. Many of the current and proposed risk assessment scenarios are worst case and are based on theoretical assumptions that certain scenarios could constitute a risk rather than based on evidence of actual risk. In July 2013 the European Food Safety Authority (EFSA) released its final guidance on the risk assessment of plant protection products (PPPs) to bees (EFSA, 2013). This scheme requires a first tier assessment through the use of a scenario which is a worst case exposure of bees through foraging on attractive weeds within the treated field. The guidance suggests, as a refinement option, that if < 10% of the area of use is covered in attractive weeds then the exposure route is not relevant in the 90th/ile case. Efficacy trials for herbicides follow common practices throughout Europe, are different in their measurement coverage in-field, and are of a standard suitable for regulatory submissions. This project aims to, using this empirical evidence, answer the question posed by the EFSA bee guidance document regarding the relevance of the weeds in the treated field scenario: “Is a significant fraction of the surface area of treated fields covered by attractive weeds for >10% of the area of use?” Using a database of around 11500 efficacy trials (containing approximately 67000 individual weed recordings) across 9 separate crop groups data was extracted on weed species, growth stage and ground cover. Initial analyses demonstrate that of all weeds recorded < 2% were at a flowering growth stage. For permanent crops this value was higher (36%) due to differences in agricultural practices. However, future analyses considering the attractiveness of flowering weeds to bees and the apparent exposure area of a bee is used, data can be used as an alternative to the HQ ratio. This new approach to measure risk is more accurate and could become a tool to aid the decision-making process in the risk assessment of pesticides.

TU315

Diversity and abundance of pollinators in chlorpyrifos-treated cider apple orchards in Herefordshire UK

C. Garrido, BeeSafe; U. Zumkier, TierSolutions; C. Wolf, TierSolutions GmbH; S. Norman, RidgewayEco; G. Weyman, Makhteshim-Agan; N. Poletka, Dow Agrosciences / RSGAACES

Though special attention is given to the protection of pollinators in agricultural crops, only little is known about effects of specific PPP applications on the non-managed pollinator communities. The aim of the study was to monitor the diversity and abundance of the pollinator populations on cider apple orchards in Herefordshire, UK, treated with chlorpyrifos. Risk to pollinators is managed by never applying during blossom-time. Ten conventionally-managed orchards were...
Cyantraniliprole residue can be found in guttation droplets of young emerging rape plants, but the cyantraniliprole concentrations in guttation droplets show a rapid decline. No residues of cyantraniliprole metabolites were detected in any rape guttation liquid samples. Worst-case oral risk assessments indicate low risk for bees resulting from the potential cyantraniliprole uptake via guttation liquid. Cyantraniliprole residues or residues of plant metabolites were not detected in pollen or nectar of flowering summer or winter rape or in bee matrices like honey or water. In vitro toxicity studies with honeybee colonies exposed next to flowering winter rape treated with DuPont™ Luminosa® showed low cytotoxicity compared to control. Bees Germany and France confirmed the safe use of DuPont™ Luminosa® and lack of any effects on honeybee colonies. Based on the available data for cyantraniliprole and its metabolites it is unlikely that the intended use of DuPont™ Luminosa® as oilseed rape seed treatment will have any unacceptable in- and off-crop effects on bees resulting from systemic exposure (guttation droplets, nectar or pollen) or from dust drift during drilling.

**TU314** Detection of morphological alterations caused by pesticides in bees collected on field

C. Nocelli, Ciências Biológicas / Departamento de Ciências da Natureza e Matemática / Departamento de Ciências da Educação; O. Malaspina, Universidade Estadual Paulista UNESP / Centro de Estudos de Insetos Sociais Departamento de Biologia; T. Roat, Biology; P. Cintra Socolowsky, Universidade Estadual Paulista UNESP / Centro de Estudos de Insetos Sociais Departamento de Biologia; O. Malaspina, Universidade Estadual Paulista UNESP

Bees are the most important pollinators of native and cultivated areas. In their activities to collect pollen and nectar they can be exposed to pesticides. Many laboratory studies have shown that different molecules causes impairment in target and non target organs, but there are no data about what can happen in these organs in bees on field. To understanding, this study aimed evaluate brain, gut and reproductive organs of bees collected on of the two study fields in native and cultivated areas. Bees were collected on flowers of the orange surroundings the Mogi Guaçu Biological Reserve and on native species. Their organs were removed, fixed in and processed to histology. The positive control were treated with dimethoate LD50 to africanized Apis mellifera. The gut from the treated and collected bees showed an increase of cells in the lumen. Epithelial cells showed increase in the secretory granules, nuclear alterations with condensed chromatin and nucleolar irregularities. There were differences in regenerative cells as degeneration and spaces between them. We were observed Malpighian tubules cells with an increase in the cellular volume, losses in the basal and apical region of the cells, and decrease of lumen diameter. In the brain no alterations were detected. The alterations observed can be linked to decreasing individual capacity to intake food and process toxic substances leading to death. These results indicate that bees can suffer effects of pesticides even in preservation areas, because the areas are small and they need to forager in crops to get food. This is a problem not just for foragers, but to all the colony that depends of them to collect pollen and nectar and can receive less and contaminated food.

**TU319** Large-scale field study of seasonal effects of Clothianidin dressed oilseed rape on pollinating insects in northern Germany: Residues in nectar and pollen collected by honey bees in tunnel tents

M. Persoghi, Tier3 Solutions GmbH

In order to investigate potential long-term side effects of Elado® (10 g Clothianidin & 2 g Beta-Cyfluthrin Ag seed) dressed Oilseed Rape (OSR) on different pollinating insects, a large-scale monitoring project was initiated in 2014 in Mecklenburg-West Pomerania, Germany. In this region, OSR is usually cultivated at 25 - 33 % of the arable land. Within this project the present residue study provided reliable data of Clothianidin residues in nectar and pollen of Elado treated and untreated OSR collected by honey bees (Apis mellifera). The study was conducted on 35 study fields of a control and a treatment site of approx. 65 km² each. During OSR blossom 73 pollen and 73 nectar samples of 18 Elado treated study fields and 17 control fields were sampled and analysed for residues of Clothianidin and its metabolites T2ZNG and T2ZMU. Between 21 April and 16 May 2017, 56 tunnel tents (in size with 500 honey bees) were set up in the study and control fields and small honey bee colonies enclosed to expose the honey bees exclusively to OSR. A minimum of 100 mg of OSR pollen was sampled via pollen traps attached to the honey bee hive. About 200 returning honey bees were sampled before entering the hive to collect nectar and their honey stomach dissected. Analyses of samples were conducted by Eurofins Agroscience Services Chem GmbH, Hamburg, Germany. Neither Clothianidin or its metabolite were present at 34 samples of nectar and pollen from the control site: residue concentrations of Clothianidin in pollen and nectar were below the Limit of Detection (LOD = 0.3 µg/kg), except of three pollen samples with Clothianidin residues of below the Limit of Quantification (LOQ = 1.0 µg/kg). In mean the metabolites T2ZNG and T2ZMU were analysed as < LOD in all pollen and nectar samples. At the treatment site, Clothianidin was detected in pollen and nectar of all study fields. In 34 out of 39 pollen samples and 22 out of 39 nectar samples of the treatment site treatment site residue concentrations were slightly higher than the LOQ. Residues of T2ZNG in pollen and nectar were lower than the LOQ or even lower than the LOD, whereas residues of T2ZMU were below the LOD in all samples. Maximum analysed concentrations of Clothianidin were 3.6 µg/kg in...
nectar and 3.5 μg/kg in pollen and on mean average 1.3 μg/kg in nectar and 1.7 μg/kg in pollen. In summary, this study constitutes a worst case scenario of honey bee exposure since honey bees collected pollen and nectar only from the target plant OSR in tunnel tents.

TU320
Sampling Methods of Nectar and Pollen of Brassica napus (oilseed rape) and Phacelia tanacetifolia (Lacy Phacelia)
S. Knueb, EAS EcoChem GmbH / Ecotox Field; P. Mack, EAS EcoChem GmbH; S. Bocksch, Eurofins Agroscience Services GmbH
The new EFSA guidance document on the risk assessment of plant protection products on pollinators includes not only the honey bees but also bumble bees and solitary bees. As a refinement agents factors were introduced for bumble bees and solitary bees to account for their potential greater sensitivity, since there are no standard test protocols available for testing. For a risk assessment refinements a more precise estimate of the possible exposure via the expected residue values in nectar and pollen is possible. With regards to the specific morphology and various yield of pollen and nectar of the different plant species it is necessary to adapt the sampling methodology accordingly since it is important to get enough material for the subsequent residue analysis. Over the last years we have conducted several studies with sampling of nectar and pollen and will present our experience with manual methods as well as with the use of honey, bumble and solitary bees exemplary for Brassica napus and Phacelia tanacetifolia. Results from an evaluation of forager bee samples for the amount of nectar and pollen load will be presented and different sampling methods will be discussed.

TU321
In vitro honey bee larval testing: lessons learned and improving the way forward
With the recently adopted OECD guideline 237 for in vitro bee larval acute testing in the laboratory, a new step towards assessment of effects on honey bee brood was implemented. Having now performed several dozen of these new studies under GLP, a second thought on current shortcomings and possible modifications of the test design is useful. Even though this new in vitro larval test may be useful for preliminary screenings on possible hazardous substances for the bee brood, from the stakeholders’ point of view, the test design is questionable and several improvements may need to be considered to increase the robustness and reliability of the test. Results for controls and reference item treatments from several data sets are presented. Recommendations on possible modification of the test design are discussed.

TU322
Variability of brood termination rates in bee brood studies according to Oomen and OECD GD 75*  
J. Lueckmann, Rifcon GmbH; R. Becker, BASF Aktiengesellschaft; S. Schmitzer, IBCON GmbH
*on behalf of the bee brood working group of the German AG Bienenschutz and the ICP-PR Bee Brood Working Group Based on EU Regulation 1107/2009/EC the current regulatory risk assessment on bees has to address the risk on honeybee larval brood. According to the new “EFSA Guidance Document on the risk assessment of plant protection products on bees” (EFSA 2014), both, the Oomen bee brood feeding test (Oomen et al., 1992) as well as the OECD Guidance Document 75 (2007; OECD GD 75) are given as the two highest tiered options to refine the risk on honeybee brood if concern is raised in tier 1. Concerning the Oomen method the EFSA GD recommends extending the feeding period of a contaminated feeding solution from one to nine days to ensure a chronic exposure to the larval stages. Hence, a suitable test design for the chronic Oomen feeding test was developed and subsequently ring-tested in 2013 and 2014 by members of the aforementioned working of the German AG Bienenschutz. Additionally results of acute Oomen feeding studies with the aim to compare the results of brood termination rates (hereafter BTR) as one of the main endpoints were compiled and assed (Lückmann & Schmitzer 2014) [1]. Furthermore, BTRs in OECD GD 75 bee brood studies displayed a certain degree of variability. Therefore Pistorius et al. (2011) [2] proposed several modifications to improve the method and their success were evaluated verified by Becker et al. (2014) [3]. The current presentation gives the updated findings on BTRs, presents the analysis of BTRs in acute and chronic Oomen GD 75 testing and finally shows the possibilities and limitations of these three methods. For this purpose data of more than 20 acute plus 7 chronic Oomen feeding studies and more than 60 OECD GD 75 studies from Germany and Switzerland were assessed. ------------------------- [1] Lückmann J & Schmitzer S (2014): The effects of fenoxycarb in a chronic Oomen feeding test – Results of a ring-test. 12th Internat Symp ICP-PR, Ghent, Belgium, 2014. [2] Pistorius J, et al. (2011): Effectiveness of method improvements to reduce variability of brood termination rate in honey bee brood studies under semi-field conditions. 11th Internat Symp ICP-PR, Wageningen, Netherlands 2011. [3] Becker R, Lückmann J & Pistorius J (2014): Effectiveness of method improvements of OECD GD 75 – Evaluation of the ICP-PR Bee Brood Working Group. 12th Internat Symp ICP-PR, Ghent, Belgium, 2014.

TU323
New methods to assess forager losses in honeybees
M. Wang, WSC Scientific GmbH / Dept Efate Modelling
In the context of colony collapse disorder (CCD) forager losses by pesticide use has been widely discussed. Currently, the extent of foragers associated to toxicants is based on a manual marking of individual honeybees, which are observed when they leave a hive, when they subsequently reach a foraging station and when they return to the hive. Since this method is rather labor intensive, we explored methods using video analysis in order to measure the number of foragers leaving the hive and returning from foraging trips. With this method large numbers of foragers can be analyzed automatically and also diurnal patterns of activity can be studied.

TU324
Reducing sampling bias in honeybee monitoring studies
M. Wang, WSC Scientific GmbH / Dept Efate Modelling
In recent years, a number of new methods have been tested to obtain large amounts of reliable, quantitative data for risk assessment of honeybees. Especially the use of digital photography has become a wide spread method to evaluate either the brood development or the development of the entire colonies under field conditions. Without efficient computer methods, the analysis of large data bases of images is not practical. Therefore, only single combs are usually evaluated manually. However, while computer methods do make it possible to analyze large quantities of data, they include a small power error. Using power analysis, we developed conventional and computer methods in order to reveal which test is more accurate and how to obtain the most reliable results from monitoring studies.

TU325
How to assess the reproductive toxicity of pesticides to the honey bee, Apis mellifera. L.?
G. Kairo, INRA (Institut National de la Recherche Agronomique), F. Ben-Alkabdeker, H. Haji, M. Cousin, Y. Poquet, S. Tchanmitchian, M. Bonnet, J. Sénéchal, M. Pelissier, A. Kretschmar, INRA; L. Belzunces, INRA / Umr Abilles Environnement; J. Brunet, INRA
By its pollinating activity, the honey bees are of high agro-environmental importance. However, this activity exposes them to myriad of environmental pollutants including pesticides. Pesticides and especially insecticides have been recently identified as a putative cause of honey bee colony losses. It has been shown that insecticides not only elicit lethal effects but also have adverse sublethal effects. They could be more insidious inducing physiological and behavioural disturbances leading to reduced reproduction. Results for controls and reference item treatments from several data sets are presented. Recommendations on possible modification of the test design are discussed.

TU326
Higher tier testing for non-Apis bees: current state of play
S. Kimmel, Innovative Environmental Services (IES) Ltd. / Ecotoxicology; C. Sandrock, Innovative Environmental Services IES Ltd / Ecotoxicology
Toxicology to colonies weighing hundreds or thousands of individual bumble bees, solitary bees, such as bumble bees and solitary bees, and there is still considerable uncertainty: either regarding extrapolating from honey bee colonies that are routinely tested but still exhibit significant declines in colony numbers during the crucial pollination period, or when the colonies are exposed to sublethal concentrations of the requested laboratory or higher tier studies in a GLP-compliant manner despite the general lack of standardisation and validation. Aside the theoretical approach of modelling the effects for non-Apis bees based on actual effects on honey bees, the setup of standardized and validated methods for higher tier non-Apis studies is of great value and will gain even more importance in the near future. Furthermore, the aspect of gathering realistic exposure residue analysis data in higher tier terrestrial studies increases in importance as well. The here presented data provides some examples conducted under GLP, the combined approach of effect testing and residue analysis and the proposal of some technical solutions as well as their advantages and limitations.
TU327
Short-term acute and extended chronic toxicity of six selected chemicals and a reference toxicant to solitary bees, honeybee and bumble bees
H. Hesketh, Centre for Ecology and Hydrology; E. Lahive, Centre for Ecology & Hydrology (NERC); M. Heard, Centre for Ecology and Hydrology; C. Svensen, CEH, Wallingford / Pollution and Ecotoxicology; J. baas, Centre for Ecology and Hydrology; A. Robinson, A.A. Horton, Centre for Ecology and Hydrology; D. Souriceon, Centre for Ecology & Hydrology
The toxicity of a range of insecticides, other pesticides and contaminants to three bee species: Apis mellifera, Bombus terrestris audax and Osmia bicornis has been assessed in chronic feeding tests. These tests used individual worker bees for the control species male and female solitary bees. The time series effects on mortality obtained from these tests was used to parameterise dynamic energy budget toxicity (DEBox) analysis tools for calculating toxicokinetic and toxicodynamic and the full time-course of effect. Chronic feeding results highlighted different pattern of effects both in the control and exposed bees. Among species there were variations in the rates of background mortality. These differences influenced the assessment of toxicity using classic concentration response models, but they can easily be accounted for within DEBox. Between chemical contrasting patterns in the time dependence of observed effects were found. For some chemicals a rapid progression of toxicity in time was seen, such that there was only a relatively small variation between effect concentrations calculated for the short and long-term exposures. DEBox analysis suggested that for these chemical internal concentration for honey bee and plant protection products (PPPs) and is used an EFSA Scientific Opinion and Guidance Document. Relevant gaps in the current testing systems, especially concerning non-Apis bees, were identified. For bumblebees (Bombus spp) several methods for toxicity testing of PPPs have been published over the last years (e.g. van der Steen 1994 and 1996). The methodologies developed for bumblebee workers in the laboratory are based on the essential methods for honey bee (Apis spp), toxicity testing and allow a determination of an acute oral and/or contact LD50. Since then the test methods have been adopted and modified several times but so far no agreed testing protocol exists. Beginning of 2014 the ICPPR Non-Apis working group, representing 13 laboratories from academia, contract research institutes and chemical industry, agreed on two draft protocols for contact (group and single housing) and one draft protocol for oral laboratory (single housing) tests with bumblebees. During 2014 tests according to these protocols were performed by several laboratories. Experimental results from 11 single contact tests, 14 group contact tests and 13 single oral tests were collected. First data show that reproducible results were obtained with the single housing methods (contact and oral) with control mortalities usually not exceeding 10%. There was a tendency for a decline of the LD50 estimates over time, which would typically slow down after 48 h. The results indicate that the test produces reliable results within 48 to 96 h. The variability in the data (LD50 values) of the different laboratories was highest for the oral exposure and the group contact exposure tests. The endpoints obtained in the single contact tests were more consistent among laboratories. The study shows that the new protocols for acute bumble bee testing are promising but additional work is needed for a validated and workable test guideline.

TU328
First results of a method proposal for a Bumblebee (Bombus spp.) first tier acute contact and oral laboratory test
N. Hanewald, BASF SE / Ecotoxicology; I. Roessink, Alterra; M. Sergey, Independent Consultant; K. Amself, BioChemagrar GmbH; L. Bortolotti, CRA-API; M. Coll, Biotechnologie BT Sri; D. Gladbach, BayerCropScience; S. Krah, Innovation Center Europe (I.C.E) / Ecotoxicology; C. Molitor, Testapi, E. Noelle, SynTech Research; H.P. Schmitt, Eurofin Agrosciences Services EcoChem GmbH / Terrrestrial Ecotoxicology Laboratory; S. Haupt, IBACON GmbH; S. Wilkins, FERA; L. Jeker, DR. KNOELL CONSULT Schweiz GmbH; S. van der Steen, WUR
The European Food Safety Authority (EFSA) has evaluated the current risk assessment for honeybee and Plant Protection Products (PPPs) and issued an EFSA Scientific Opinion and Guidance Document. Relevant gaps in the current testing systems, especially concerning non-Apis bees, were identified. For bumblebees (Bombus spp) several methods for toxicity testing of PPPs have been published over the last years (e.g. van der Steen 1994 and 1996). The methodologies developed for bumblebee workers in the laboratory are based on the essential methods for honey bee (Apis spp), toxicity testing and allow a determination of an acute oral and/or contact LD50. Since then the test methods have been adopted and modified several times but so far no agreed testing protocol exists. Beginning of 2014 the ICPPR Non-Apis working group, representing 13 laboratories from academia, contract research institutes and chemical industry, agreed on two draft protocols for contact (group and single housing) and one draft protocol for oral laboratory (single housing) tests with bumblebees. During 2014 tests according to these protocols were performed by several laboratories. Experimental results from 11 single contact tests, 14 group contact tests and 13 single oral tests were collected. First data show that reproducible results were obtained with the single housing methods (contact and oral) with control mortalities usually not exceeding 10%. There was a tendency for a decline of the LD50 estimates over time, which would typically slow down after 48 h. The results indicate that the test produces reliable results within 48 to 96 h. The variability in the data (LD50 values) of the different laboratories was highest for the oral exposure and the group contact exposure tests. The endpoints obtained in the single contact tests were more consistent among laboratories. The study shows that the new protocols for acute bumble bee testing are promising but additional work is needed for a validated and workable test guideline.

TU329
Experiences with bumble bee semi-field and field studies for ecotoxicological risk assessment
O. Klein, T. Juette, G. Bosse, Eurofin Agrosciences Services EcoChem GmbH / Ecotoxicology Field
Since many years, semi-field greenhouse studies are performed with bumble bees (Bombus terrestris) to assess eventual impacts on pollination efficiency. The new proposed EFSA risk assessment of plant protection products for pollinators includes for the first time not only honey bees but also non-Apis pollinators. However, no official guideline for the testing of non-Apis pollinators in semi-field and field trials exists so far. To overcome this lack of guidance suitable semi-field and field test designs have to be developed. Experiences on different designs for greenhouse, semi-field and field studies are presented. Possible endpoints and techniques to assess these are discussed focusing on different attractive crops like phacelia and oil seed rape and less attractive crops like tomato, potato and cucurbitaceous plants. Endpoints including adult and larval mortality, flight and foraging activity, hive weight development, food consumption and reproduction (worker, queen and drone production) are considered. The aim of this presentation is to give an overview on possible test designs, their chances and limitations.

TU330
First results of a method proposal for a solitary bee (Osmia spp.) first tier acute contact laboratory test
I. Roessink, Alterra; N. Hanewald, BASF SE / Ecotoxicology; C.W. Schneider, BASF SE; P. Medrzycki, CRA-API; J. Bosch, University of Barcelona; S. Hinarojos, Sumitomo Chemical Agro Europe SAS / Registration / Regulatory Affairs; D. Gladbach, BayerCropScience; L. Stanisavljević, University of Belgrad;
Variable effects of As(V) and carbon source on nutrient stoichiometry, pigments and phenolic...

showed significant affects starting at 7.2 ppm A

allocation (leaf mass fraction / LMF, root/shoot ratio / R/S of factorial design we exposed arsenate toxicity toward the latter being the dominant carbon source at alkaline pH. We hypothesize systems, exhibiting a broad environmental tolerance e.g. to pH, nutrient and carbon...

showed that the new protocols for acute Osmia spp. testing are promising but additional work is needed for a validated and workable test guideline.

TU333 Development of suitable experimental designs for field and semi-field trials with the solitary bee Osmia bicornis

The new proposed EFA risk assessment of plant protection products for terrestrial plants (P) were conduct for trials under field and semi-field conditions with solitary wild bees. However, no official guideline for the testing of nonApis pollinators in semi-field and field trials exists so far. To overcome this lack of guidance a suitable field test design has to be developed and ring tested. Also there is more basic knowledge needed as a foundation for field testing. Semi field and field studies were performed this year to increase the knowledge about suitable test designs and the handling of the test organisms. The aim of our studies was the development of a test system for trials under field and semi-field conditions with solitary wild bees. The species chosen for the test was Osmia bicornis as proposed in the SETAC / ICP-PR approved guideline on semi-field and field trials with Osmia bicornis were conducted in two different crops. For this purpose winter oil seed rape and Phacelia were chosen. Different nesting materials, set-ups and release techniques were tested. First results on the hatching success, dispersal rates, acceptance of different nesting materials, the nest occupation by female individuals, the reproduction capacity by means of produced cells and cocoon will be presented and discussed. Recommendations are given on possible test designs.

From ecosystem services to risk assessment for aquatic and terrestrial plants (P)

TU334 Sensitive response of sediment-grown Myriophyllum spicatum exposed to carbon limitation and environmentally relevant arsenate pollution

E.M. Gross, University of Lorraine / Laboratoire Interdisciplinaire des Ecosystèmes Continentaux; D. Paroshin, University of Lorraine / LIEC CNRS UMR; A. Hussner, Heinrich-Heine University Düsseldorf Myriophyllum spicatum has recently been included as new test system for rooted aquatic plants (OECD239). This submerged macrophyte is found in diverse aquatic systems, exhibiting a broad environmental tolerance e.g. to pH, nutrient and carbon availability. The plant can use both CO2 and bicarbonate for photosynthesis, the latter being the dominant carbon source at alkaline pH. We hypothesized that arsenate toxicity toward M. spicatum would be stronger when the plant has to rely on bicarbonate. Also, plants grown under carbon depleted conditions grow better with CO2. In a 2 x 4 factorial design we exposed M. spicatum to either CO2 (high carbon or HC) or bicarbonate (low carbon or LC; pH 6.8 and 8.1, respectively) and 0 – 1.44 and 36 ppm As( V) in the sediment. Plants were grown for 2 weeks in a set-up adapted from the new OECD239 guideline. We measured different endpoints based on growth (length, fresh and dry mass, relative growth rates / RGR), biomass accumulation (leaf mass fraction / LMF; root/shoot ratio / R/S) and physiological parameters (CNP stoichiometry, pigments, phenolic compounds). Most endpoints showed significant affects starting at 7.2 ppm As(V), with mostly no difference between 0 and 1.44 ppm and 7.2 and 36 ppm As(V). Growth based on RGR-L and dm, and R/S-r declined with higher As(V) concentrations, but carbon source affected only RGR but not R/S-r. LMF declined already at the lowest As(V) concentration, and was less affected at LC. Nutrient (N, P) content was affected only by the treatment in the whole plant and in leaves, but little affected by carbon source. Variable effects of As(V) and carbon source on nutrient stoichiometry, pigments and phenolic compounds were observed, often showing the interaction of both factors for a given endpoint. We conclude that the new test guideline for M. spicatum can be used in modified set-up to evaluate the role of resource availability (CNP) on pollution effects. Such modifications are useful to establish variable effects due to environmental heterogeneity, i.e. based on nutrient availability or carbon source such as observed in our study.

TU335 Use of sanitized sewage sludge as agricultural input: Assessment of biochemical, morphological, anatomical and cytogenetic effects in Carica papaya L. and Allium cepa L.

M.M. Marques Bonomo, Federal University of Sao Carlos / Department of Physiological Sciences; M. Morozes, UFSCar / Departamento de Ciências Fisiológicas; Ld. Souza, Federal University of Sao Carlos / Departamento de Ciências Fisiológicas; A. Marques, Federal University of Sao Carlos / Departamento de Ciências Biológicas; L. Rocha, I. Duarte, Universidade Federal do Espirito Santo / Departamento de Ciências Biológicas; A.d. Costa, Federal University of Espirito Santo / INCAPER; M.N. Fernandes, Universidade Federal de Sao Carlos / Ciências Fisiológicas; S. Matsumoto, Universidade Federal do Espirito Santo Sewage sludge is a biosolid residue which final destination represents an environmental concern. Its growing use as agricultural input is an effective measure, contributing in biogeochemical nutrient cycle and acting as an additional source of organic matter, micro and macro nutrients thus stimulating plant growth. Thereby, plant growth assessments allow in monitoring individual development and the effects in each organ, representing a very useful tool to compare distinct situations. In addition, evaluations of biotransformation and antioxidant enzymes associated to cytotoxic analysis are broadly applied in monitoring of compound effects. Therefore, the aim of this study was to evaluate the effects of sewage sludge application in initial growth of Carica papaya and its cytotoxic influences in Allium cepa, integrating chemical characterization and biological responses to elucidate potential risks of this residue utilization. Nutritional levels in chemical analysis of substrates corroborates the potential of sanitized sewage sludge use as soil conditioner and additional nutrient input, as well as the low levels of analyzed contaminants make the residue within established by Brazilian legislation. High enzymatic activity in substrate without sewage sludge addition, after 60 and 90 days, confirmed the nutritional deficit promoted by the lack of soil supplementation which may lead to a high metabolic cost to plants. Furthermore, sanitized sewage sludge addition stimulated plant development which was evidenced by the higher growth in plants with higher residue doses applied. Moreover, biochemical and cytogenetic assessments also complements results observed in growth evaluation of C. papaya in order to help the determination of ideal application rate, where genotoxic and mutagenic effects were detected in higher doses, along with first biochemical results. Thus, findings of this study indicates that an excessive addition of sanitized sewage sludge may prevent the responses of plants in order to adapt to these stress conditions, which can lead to damage generation, where balancing costs and benefits of this residue application are essential to assure a high crop quality and help in environmental management.

TU336 Toxicity tests with 3,5-dichlorophenol on six aquatic plant species in water-sediment system

H. Rzodeczko, Institute of Industrial Organic Chemistry Branch Pszczyna / Dpt of Ecotoxicology; K. Brzozowska, Institute of Industrial Organic Chemistry Branch Pszczyna; A. Swierkot, Institute of Industrial Organic Chem. Branch Pszczyna; P. Fochtman, IPO Pszczyna Aquatic plants toxicity testing plays a role in risk assessment of various compounds. Water-sediment system, considered as ecologically relevant model of toxicity testing, was used in studies conducted with the reference substance recommended in standard tests (OECD TG 201, 221, 239). 3,5-dichlorophenol (3,5-DCP) interferes with oxidative phosphorylation process and algae, duckweed and aquatic macrophytes are sensitive to its mode of action. Results of the toxicity tests performed with six species of freshwater plants were compiled. These were with - dicotyledonous rooted species: eurasian watermilfoil (Myriophyllum aquatimum) and parrot’s feather watermilfoil (Myriophyllum spicatum), - monocotyledon rooted species: canadian waterweed (Elodea canadensis), curly waterweed (Lagarosiphon major) and dense waterweed (Egeria densa), - monocotyledonous floating species: duckweed (Lemna gibba).

TU337 Plant density and growing conditions in NTTP studies - influence on data validity

H. Tresiak, C. Stroemel, Agro-Check For testing phytoxicity of non-target terrestrial plants (NTTP) in the context of plant protection products (PPP) registration processes in Europe two study guidelines are currently in use: Seeding and early growth test (OECD 208) and vegetative vigor test (OECD 227) Plant biomass - the key evaluated endpoint - is dependent on equal seedling emergence and evenness during the following growth process. Therefore the validity criterion for NTTP studies, an emergence rate of ≥ 70%, was set. Within both OECD guidelines in addition seed densities of 3 - 10 seeds per 100cm², dependent upon the species, pot size and test duration, are recommended, to secure adequate growth conditions and to avoid any overcrowding during the study conduct. This is a main factor to determine significant differences. There have been concerns raised about the value of NTTP...
studies, some focusing on potential overcrowding in the test pots. From the perspective of a testing facility, we want to present information demonstrating the influence of plant densities on emergence and plant growth. Seed viability of test species used in NTTP studies varies due to inherent species characteristics, dormancy pattern and also with the degree of breeding progress. Improved seed viability is one advantage of using crop species. Cereals, maize and tomato normally reach higher seed viability than other crop species like carrot or onion have generally lower viability (75-80%), although only high quality seed batches are used. We also give information how an equal emergence will influence the further growing process and therefore the significance of the test results. We will discuss how seed viability may influence plant density in the pots. We present some generic data from our lab to compare effects of different plant densities and will discuss a range of parameters influencing plant growth and how these parameters can be controlled to prevent effects that could be linked to potential overcrowding. Further we will also discuss pragmatic options for the implementation of recommendations laid out in the OECD Guidelines 208/227 used for phytotoxicity testing.

TU338 Prioritizing Ecosystem Services for Risk Assessment: Whose Values Count? Y. Pan, University of Sheffield / Animal and Plant Sciences; L. Maltby, The University of Sheffield / Dpt of Animal Plant Sciences

It is impossible to protect everything in the environment and a key question for decision makers is what do we want to protect? One approach is to base protection goals on the values of society. Identifying ecosystem services (i.e. benefits that we receive from ecosystems) that are important to society can help decision makers to prioritize specific services for protection. The specific protection goals prioritized can then be used to guide environmental risk assessments. Understanding which ecosystem services are valued by different sections of society is crucial to establishing effective specific protection goals. For example, do rural and urban communities prioritize different ecosystem services?

TU339 Evaluation of non-crop wild species with respect to their suitability in regulatory testing of PPP H. Teresiak, Agro-Check; C. Mayer, BASF SE / Ecotoxicology; M. Geiger, BASF SE / Crop Protection

Introduction and Method The use of wild species as test species is currently discussed for NTTPs and for certain PPP purposes. How? If not, OECD 208/227 – Seeding emergence/Vegetative Vigor) include a list of non-crop wild species (NCWS), which were considered suitable in meeting the validity criteria for testing in the scope of these guidelines. However, suitability of these species has not been subject to systematic scrutiny. The above mentioned validity criteria include ≥70% emergence rate and low variation in growth and morphology. Further criteria for selection of test species include constant availability of uniform high quality seeds, displaying reliable and even germination among different seed batches, further species must be compatible with growth conditions of the test method. NCWS seeds can be obtained commercially including information on their viability; latter however is often based on different methods, some including the use of germination paper/tissue methods, which are not allowed to be used in OECD studies. Seed batches of nine selected NCWS (8 families) from different sources were tested on their germination and growth behavior to evaluate their suitability in phytotoxicity studies according to OECD 208/227. Bioassays comprise parallel sets of germination tests on filter paper and emergence/growth tests in soil. Each set of bioassay was conducted at three different periods within one year (Jan/Feb, May/Jul, Sep/Oct) to investigate potential seasonal effects. The emergence rates were less than germination rates and seed viability information did not match the experimentally determined germination rates. Only few species reached 70% emergence in the tested batches and many species show seasonal influence on germination and emergence rate. A high variability in emergence and seedling vigor increases variance of endpoint biomass which impedes a robust statistical analysis. Conclusions Most of the tested NCWS do not meet the required quality criteria with respect to emergence rate, seedling vigor and equal plant development. Only one species showed reliable germination rates independent of batch and season, however, failed concerning the parameter ‘growth characteristics’. Three further species meet the emergence rate criteria in a limited amount of seed batches. Although a range of NCWS are listed as potential test species in respective OECD guidelines, they may not be suitable due to low quality seed material.

TU340 Primary producers in pesticide risk assessment: endpoints and level of protection K. Freytag, German Federal Environment Agency (UBA)/ Dept IV; S. Duquesne, UBA, Federal Environment agency; L. Hönnemann, Umweltbundesamt Federal Agency of Environment; S. Matezki, Plant Protection Products; U. Kühnen, Federal Environment Agency Germany; A. Aldrich, Research Station Agroscope ACW / Ecotoxicology; J. Berchtold, Austrian Agency for Health and Food Safety / Institute of Plant Protection Products Department for Ecotoxicology Efficacy and Integrative Pest Management (IBW); V. Tighzert, ANSES, French Agency for Food, Environmental and Occupational Health Safety; P. van Vliet; J. Wogram, German Federal Environment Agency UBA / Section Plant Protection Products

As part of the current risk assessment of plant protection products towards aquatic organisms, the risk for primary producers is assessed using EC₅₀ (growth inhibition) as endpoint. EC₅₀ can be expressed as inhibition of the average specific growth rate (EC₅₀) or as reduction biomass, calculated from final biomass (EC₅₀) or as the integral under the growth curve (EC₅₀). Current PPP data requirements (Annex III to Regulation (EU) No 544/2011) do not specify which EC₅₀ should be used. Hence, according to the precautionary principle, for past regulatory decisions, the lowest available figure (usually EC₅₀) was used to derive safe concentrations of pesticides in surface water bodies. The new aquatic guidance document (EFSA, 2013) recommends setting the EC₅₀ on the level of protection when shifting from a biomass to a growth rate endpoint. These data indicate that using growth rate endpoints systematically lowers considerably the level of protection of current risk assessment, i.e. by a factor 4.5 and 9.5 (90% percentile for Algae and Lemma, respectively). According to the EFSA Opinion on the development of specific protection goals options for aquatic risk assessment (EFSA, 2010), new risk assessment methods according to regulation 1107/2009 should be calibrated to meet specific protection goals, which was not done yet for aquatic primary producers. As a conclusion, we strongly recommend to calibrate the current PPP risk assessment for primary producers to ensure that lowering the level of protection will however meet the intended level of protection.

TU341 Effect response of Myriophyllum spicatum - Extrapolating chemical induced effects from the lab to the field S. Heine, Bayer CropScience / Environmental Modelling; G. Goerlitz, Bayer CropScience AG / Environmental Safety; E. Bruns, Bayer CropScience AG / BCS D / CropScience / Environmental Modelling; T. J. Preuss, Bayer CropScience AG / Environmental Modelling; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences

The prediction of environmental concentrations (PEC) of chemicals, such as plant protection products occurring in edge-of-field water bodies, is a basic principle of the risk assessment. Therefore, long-term exposure patterns of specific chemicals are needed which are relevant on the level of protection when shifting from a biomass to a growth rate endpoint. These data indicate that using growth rate endpoints systematically lowers considerably the level of protection of current risk assessment, i.e. by a factor 4.5 and 9.5 (90% percentile for Algae and Lemma, respectively). According to the EFSA Opinion on the development of specific protection goals options for aquatic risk assessment (EFSA, 2010), new risk assessment methods according to regulation 1107/2009 should be calibrated to meet specific protection goals, which was not done yet for aquatic primary producers. As a conclusion, we strongly recommend to calibrate the current PPP risk assessment for primary producers to ensure that lowering the level of protection will however meet the intended level of protection.


Recent guidance on the conduct and interpretation of mesocosm tests highlights the importance of using the most appropriate aquatic communities for the chemical
being investigated. In addition, it is equally important that for robust effects
assessments, the selection of appropriate measurement endpoints and ecological
evaluations, is critical to the derivation of powerful statistical results with
reasonable Minimum Detectable Difference (MDD) values. Furthermore, selecting
an experimental design aligned with the endpoints of interest has been identified as a
factor that might significantly increase the statistical power of experimental
studies for risk assessment. Here we will show the evolution of study design and
endpoint selection for macrophytes using examples drawn from recent state of the
art regulatory tests with herbicides. Earlier studies were conducted using flat
bottomed mesocosms that included deep water macrophytes planted into the
sediment whereas shallow water and marginal plants were potted and suspended at
varying depths within the water column. More recently, we have conducted mesocosm
studies with herbicides using sloped mesocosms that enable macrophytes to be planted at water depths that most accurately reflect natural edge of field water-bodies allowing for true community effects to be evaluated. We will
provide an analysis of the available data for macrophytes comparing the respective
reliability of the data generated at each stage of development (mesocosm design and
effects measurement parameters) for both mesocosm designs. Additionally, we will
also provide recommendations for further work in order to still improve the
robustness of the endpoints for use in regulatory risk assessment.

**TU343**

**Glyceria maxima, Ceratophyllum demersum, Cabomba caroliniana**

**Water/Sediment Tests**

G. Consier

Some plant protection products and industrial chemicals show an unavoidable high
risk for aquatic plants. Due to the fact that Lemna only reflects the risk assessment
of free-floating aquatic plants there was a need for the establishment of other
aquatic macrophyte systems. Up to now further studies could be conducted based
on the OECD 239: Sediment-free Myriophyllum spicatum Toxicity Test or OECD 239:
Water-sediment Myriophyllum spicatum Toxicity Test. However, it is still under
discussion if Myriophyllum and Lemna species could cover all questions for
doing refined risk assessment on macrophytes. Tests with Glyceria maxima are
under discussion and could be performed in addition to reduce uncertainties.
Further water-sediment tests with Cabomba caroliniana and Ceratophyllum
demensum based on OECD 239 and OECD 221 where developed and will be
presented.

**TU344**

**Myriophyllum recovery and pulsed exposure tests**

G. Consier

Up to now, tests with Myriophyllum spicatum were performed in an unsterile
water-sediment or axenic sediment-free system. Both test designs were submitted
for OECD evaluation and resulted in the OECD 238: Sediment-free Myriophyllum
spicatum Toxicity Test and OECD 239: Water-sediment Myriophyllum spicatum
Toxicity Test. However, when standard laboratory tests are resulting in an
unavoidable high environmental risk, aquatic higher tier tests are needed to reduce
uncertainties. Tests like monospecies plant tests with a toxicity phase followed by
a recovery phase are a tool for demonstrating more realistic exposure scenarios and
for doing refined risk assessment. The contribution will present data of a
water-sediment Myriophyllum spicatum toxicity test followed by a recovery phase
and a water-sediment Myriophyllum spicatum pulsed exposure test.

**Improving approaches for difficult non-standard risk assessment of chemicals (P)**

**TU345**

**Risk assessment of difficult substances under the REACH Substance evaluation process**

J. Caley, European Chemicals Agency - ECHA / Evaluation; C. Carlson, European
Chemicals Agency - ECHA; M. Sobanska, European Chemical Agency; F.
Pellizzato, European Chemicals Agency; L.A. Walker, Centre for Ecology &
Hydrology; L. Wollenberger, European Chemicals Agency

Under REACH, the responsibility for the generation of data and the chemical safety
assessment of industrial chemicals lies with the manufacturers or importers of the
substance (Registrants). Amongst the 6,600 unique substances that are prioritised based
on risk concerns. These substances are assessed by Member States under the substance evaluation process. Further information from Registrants may be requested under this process to clarify risk concerns. The list of substances to be evaluated by Member States is called the Community Rolling Action Plan (CoRAP), and includes mono- or multi-constituent substances and the so called UVCBs (substances of Unknown or Variable composition, Complex reaction products or Biological materials). By autumn 2014, 201 substances had been included in the CoRAP for evaluation between 2012 and 2016 and a significant proportion of those are UVCBs or substances that form transformation products. These substances pose particular challenges in the assessment of their PBT properties. The prioritisation for CoRAP combines hazard criteria (e.g. known or suspected PBT, vPvB and PBT like substances, suspected endocrine disruptors, known or suspected CMRs, known or suspected sensitizers) together with exposure criteria (e.g. wide dispersive use, consumer use and

**TU346**

**REACH Substance evaluation of a UVCB: assessment of PBT properties**

F. Croci, European Chemicals Agency - ECHA / Evaluation; C. Carlson, European

Testing of UVCBs for potential PBT or vPvB properties poses particular challenges due to the many different constituents they are composed of. In addition, difficulties in the analytical characterization of individual substances and their ability to also turn out to be PBT or vPvB. Therefore, one of the main challenges is to decide what to use as the test item. The approach taken will depend, e.g., on the type of UVCB being investigated. The PBT testing usually starts with the investigation of the P criterion. Once this is confirmed, the testing strategy moves to the investigation of the B criterion, and, if this is also confirmed, of the T criterion. In this poster, the main challenges of the assessment of UVCB substances will be highlighted by
providing an example of an approved PBT testing strategy under the substance evaluation process (background information for this process will be provided in the
poster entitled “Risk assessment of difficult substances under REACH substance evaluation process” also submitted to this session) evaluated by the Danish Competent Authority. The substance is a UVCB with 5 major groups of
constituents. The available information on PBT properties for each major fraction of
the UVCB was assessed. Representative structures of each major fraction were used
to conduct QSAR predictions for the following endpoints: water solubility, vapour
pressure, Log Kow, photo transformation in air, ready biodegradability, Log Koc, Henry’s law constant, fish BCF (bioconcentration factor) and short- and long-term aquatic toxicity. A tiered testing approach has been developed in order
to derive adequate information to conclude on the PBT properties. The tiered strategy
developed for this substance unusually consists in the assessment of the B criterion
first for four fractions. Constituents that are concluded to not meet the criteria for
B- or vB-based on the results from this study do not need to be investigated for their
recovery potential (for the purpose of PBT assessment). Due to the expected difficulties in the analytical characterisation of individual constituents which result in a low recovery and low statistical power of the tests in the test medium in a biodegradation simulation study, it was considered that a
dietary bioaccumulation study is more likely to provide useful results. Hence, it
may be possible to reduce or totally eliminate the number of constituents to be
assessed at tier two in the testing strategy which would involve biodegradation
simulation testing.

**TU347**

**Assessing the bioaccumulation potential of Gas to Liquid (GTL) Fuel**

G.F. Whale, Shell Health / Shell Health; J. Dawick, Shell Health / Shell Health Risk
Science Team; C.V. Eadsforth, Shell International

GTL Fuel (“Distillates (Fischer-Tropsch), C8-C26 branched and linear”) is
considered to be a UVCB (Unknown, of Variable Composition, or of Biological Origin) substance under the EU REACH definitions. This has a significant impact on how bioaccumulation potential can be assessed. In the initial testing proposals to assess bioaccumulation potential under REACH notification requirements a standard OECD 305 fish bioconcentration test on specific C13 or C14 radio labelled hydrocarbon constituents is required. The justification behind this would provide data to strengthen the Quantitative Structure Activity Relationship (QSAR)
models used to predict the BCFs (bioconcentration factors) of constituents of the
GTL Fuel. This was rejected by the European Chemicals Agency (ECHA) who
recommended that the constituents to be used in the bioaccumulation testing should
be based on the outcome of soil biodegradation testing. The latter studies raised
some questions because of analytical constraints, notably the sensitivity of the
analytical methods. Although no quantifiable persistent constituents could be
determined in soil, it could not be verified whether some constituents remained in
the soil at concentrations > 0.1% w/w of the GTL Fuel. As it was not possible to
provide ECHA with the information requested additional studies have been
undertaken and these provide weight of evidence that GTL Fuel does not have
significant potential to bioaccumulate. Furthermore, there is no evidence for
undertaking any further bioaccumulation assessments of GTL Fuel is also presented
and discussed.

**TU348**
Assessing Complex Substances: What we need and what we get

E. Burkhardt, Federal Environment Agency (UBA); D. Einhenkel-Arle, D. Claßen, German Federal Environment Agency UBA

Under the REACH Regulation substances of very high concern (SVHC), like e.g. persistent, bioaccumulating and toxic (PBT) substances, very persistent and very bioaccumulating (vPvB) substances, and substances of equivalent concern are to be identified, included in the candidate list and phased out to minimize the risks posed by these substances. Many substances registered under REACH are substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs), which cannot be sufficiently described by their chemical composition. One example for UVCB substances are substances originating from the petroleum industry. The German Federal Environment Agency (Umweltbundesamt UBA) is working on the assessment and regulation of certain UVCB substances. The strategy of the German Federal Environment Agency for the SVHC-assessment of UVCB substances is presented by two examples. To assess the hazard associated with an UVCB substance at first each constituent of the substance with at least 0.1 mass % is screened. The constituent(s) with the most severe properties with regard to SVHC is chosen for the SVHC-assessment of the substance. A substance that contains a SVHC with a fraction of at least 0.1 mass % is regarded as a SVHC itself and can be identified as such. Thus, the knowledge of the identity of the components of an UVCB substance is a prerequisite for any subsequent hazard and risk assessment. In practice several challenges are associated with this work. What we would need would be i) proper identification of all constituents of the substance with a fraction of at least 0.1 mass %, i.e. at least its name, CAS number, a specific REACH Annotation in VII – A and further interpreted in the ECHA Guidance document R.7.b[1]. The Guidance explains also the recommended integrated testing strategy for aquatic toxicity endpoints. According to REACH standard information requirements, long-term aquatic toxicity testing on invertebrates (preferably Daphnia) and fish is required for substances manufactured or imported in quantities of 100 tonnes or more. Long-term studies may be needed at lower tonnage, but then the hazard assessment is less transparent. As explained in ECHA Guidance R.7.b aquatic long-term testing on only the most sensitive trophic level may be possible if there is compelling evidence suggesting that one toxic level (fish or Daphnia) is significantly more sensitive than the other. Furthermore, if the results of the available short-term toxicity data (acceptable experimental, QSAR or read-across data) for algae, Daphnia and fish indicate that no one species is substantially more sensitive than the other, aquatic invertebrates are to be tested first. This allows the avoidance of long-term vertebrate testing (fish) in a number of cases, if the resulting risk is adequately controlled (RCR is < 1) with an assessment factor (AF) of 50 (assuming that data on algae are available). The application of this integrated testing strategy may encounter a number of diverse scientific issues that can be illustrated with examples. "<clear>="all" > [1] Guidance on information requirements and chemical safety assessment, Chapter R.7.b: Endpoint specific guidance

TU349

Integrated testing strategy for aquatic PNEC derivation under REACH


Reaching and reading across REACH Annexes VII – A and further interpreted in the ECHA Guidance document R.7.b[1]. The Guidance explains also the recommended integrated testing strategy for aquatic toxicity endpoints. According to REACH standard information requirements, long-term aquatic toxicity testing on invertebrates (preferably Daphnia) and fish is required for substances manufactured or imported in quantities of 100 tonnes or more. Long-term studies may be needed at lower tonnage, but then the hazard assessment is less transparent. As explained in ECHA Guidance R.7.b aquatic long-term testing on only the most sensitive trophic level may be possible if there is compelling evidence suggesting that one toxic level (fish or Daphnia) is significantly more sensitive than the other. Furthermore, if the results of the available short-term toxicity data (acceptable experimental, QSAR or read-across data) for algae, Daphnia and fish indicate that no one species is substantially more sensitive than the other, aquatic invertebrates are to be tested first. This allows the avoidance of long-term vertebrate testing (fish) in a number of cases, if the resulting risk is adequately controlled (RCR is < 1) with an assessment factor (AF) of 50 (assuming that data on algae are available). The application of this integrated testing strategy may encounter a number of diverse scientific issues that can be illustrated with examples. "<clear>="all" > [1] Guidance on information requirements and chemical safety assessment, Chapter R.7.b: Endpoint specific guidance

TU350

Transformation/dissolution testing used in a grouping and read across approach to determine the environmental classification of alloy brazing pastes


Umicore Technical Materials has a brazing paste and flux portfolio of +30 products. These products are mixtures of metallic alloy powder(s) (containing e.g. Ag, Cu, Zn, Ni . . . ), a binder and a solvent, of which the metallic powders form the basis of potential environmental concern. For the purpose of aquatic hazard assessment under the Globally Harmonized System of Classification (GHS), an integrated testing strategy (ITS) including grouping and read across based on ionic release using the Transformation/Dissolution protocol (TDP) was developed. Since alloys are considered to be “special mixtures” of which the functional and hazardous properties are different from those of the individual metals, grouping purely based on composition would not be valid. The multi-dimensionality of the issue was addressed by accounting for product specific properties such as type of alloy and its metal content, additional metal powder(s), and type of binder. Our general hypothesis is that “Grouping and read across can be applied for products with the same alloy type in different binders” The hypothesis is assessed in a tiered approach on a group of 7 products (Ag-Cu alloys). In a first step, TD tests are being conducted on two brazing products with the same alloy type and identical metal concentrations (Ag 72%-Cu 28%) but with a varying binder. Reason for testing is two-fold: 1) assess the behavior of the individual metal compounds in the alloy and 2) assess the hypothesis that a similar degree of hazard can be expected for similar alloy in different binders. Regarding the read across of relevant trace metal compounds, the preliminary results indicate that there is no need for a worst case environmental classification as acute 1 / chronic 1 by applying the classification rules for mixtures based on composition. Indeed, although the high Ag content (72% vs. 28% Cu) was expected to be the driver for classification, surprisingly it was release of Cu ions that triggered the less stringent aquatic acute category 3 classification. Already these first results confirm that alloys should be considered as “special mixtures” that require a dedicated approach to avoid overly conservative classification. In a next step, the impact of varying binders will be assessed. This binder assessment will be the critical step in determining the validity of this first grouping and read across exercise.

TU351

Environmental classification of coal and its implications with regard to transport regulation

D. Hejervick, ARCHE; A. Tomczak, World Coal Association; S. Hubbart, Rio Tinto; M. van Gheuwe, ARCHE

Transport of coal in bulk cargo is regulated in the past under the International Maritime Solid Bulk Cargo Code (IMSBBC Code) which classifies coal as a Group B Substance. However, newly introduced regulations (MARPOL, revised IMSBC Code), require the assessment of overseas transported substances against environmental and human health criteria that were not taken into account under IMSBC, and a classification resulting from this assessment may have significant impact on the transport and loading/unloading of the substance in question. According to the International Convention for the Prevention of Pollution from Ships (MARPOL, Annex V), on of the criteria to consider a substance as Harmful to the Marine Environment (SMHE) when it is classified as Aq.Acute Toxic 1 and/or Aq.Chronic Toxic 1.2 (environmental endpoints). Endpoint evaluation should be conducted according to the Guidance given in the Globally Harmonized System of Classification and Labelling (GHS). GHS criteria are considered in the revised IMSBC code which determines whether a substance is a Hazardous Material only in Bulk. Coal is a complex mixture of ubiquitous organic and inorganic compounds, each with their own environmental classification. This degree of complexity makes it virtually impossible to derive a meaningful classification that is based on classification mixture rules as outlined under GHS. The most relevant coal components that could trigger an environmental classification are trace metals and polycyclic aromatic hydrocarbons. A review of available coal composition data resulted in a relevant min/max range of the most relevant trace metals in coal. This information formed the basis for a set of critical limit concentrations: a coal sample that does not exceed any of these limit values, will not be classified as Aq.Chronic 2 (or more severe) for the environment, even under the worst-case scenario where worst trace elements are present in coal and present under their most toxic form. Analysis of available data on PAH-content of coal samples showed that concentration levels are several orders of magnitude below the concentration limits that would trigger an environmental classification. Both conclusions on PAHs and trace elements are confirmed by the absence of any ecotoxicological effects in tests that used unburnt coal as test substance.

TU352

The Globally (Un)Harmonised System for Classification and Labelling of Chemicals (GHS)

J. Zhang, Gradient Corp; A.S. Lewis, Gradient Corporation; K. Reid, T. Legalowski, T.A. Verkade, Gradient Group

In 2003, the United Nations (UN) published the Globally Harmonised System for Classification and Labelling of Chemicals (GHS), incorporating “harmonised criteria” for identifying chemical hazards and requirements for labeling and safety data sheets (SDS). In its current revision, GHS incorporates 16 physical, 10 health, and 2 environmental hazard categories. Various jurisdictions around the world have chosen to adopt this UN framework. Jurisdictions such as the European Union, Australia, China, and Brazil have fully implemented the GHS, while the United States and Canada are currently in the midst of implementation. To further complicate this process, the GHS model has been interpreted in several different ways: some countries have adopted the GHS “as is”, some have incorporated the criteria approach into existing legislative frameworks, and others have altered the GHS criteria to fit their existing legislative framework. This has resulted in a rapidly changing global landscape of environmental regulations and chemical compliance, presenting a major challenge for chemical manufacturers and importers. For example, selective adoption of the GHS framework can lead to a difference in hazard classifications for the same chemical or product, confidential...
business information (CBI) requirements, and legal recourse. In addition, GHG hazard classification is complex and calls for a level of expertise in (eco)toxicology and chemistry that was not previously needed to communicate chemical safety, particularly for UVCBs (Substances of Unknown or Variable composition, Complex reaction products or Biological materials). This presentation highlights some of the technical and business challenges associated with the ongoing implementation of GHS. Specifically, using several examples, we illustrate key factors that can result in different hazard classifications for the same chemical or product, such as differences in jurisdictional GHS adoption, variable supplier information, reliance on different sources, use of surrogate data, presence of impurities, and different approaches to evaluating the weight-of-the-evidence.

TU353 Key variables for dynamic modelling of persistent PBT and vPvB substances in the environment: Application in socio-economic analysis (SEA) under the REACH Regulation.

O. Warwick, Peter Fisk Associates Limited; S. Gabbert, Wageningen University / Social Sciences; P.R. Fisk, Saxon House; I. Hober, Agroscope ART; D. Disley, C. Gibson, Peter Fisk Associates Limited

Substances that are persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) attract special consideration under REACH, because no safe threshold for environmental risk assessment can be established. For PBT and vPvB substances, for which continued use depends upon the granting of an authorisation application, a socio-economic analysis (SEA) must be presented as part of a TDI application. The SEA needs to consider two scenarios: the "as used for" (AFU) and the "non-use" (NU) in which the authorisation is refused. The SEA must consider the net impacts between these two scenarios in order to decide if the benefits outweigh the risks. For the consideration of environmental impacts in the SEA, it is essential to understand how much of the substance and at what concentrations it will be in the environment at any time in the post-commissioning period. This must include consideration of the changes in the amount and concentration of the substance over time (i.e. the dynamics of the stock pollutant) since that affects the period over which impacts are relevant. In this poster we apply (a previously described) simple dynamic model for predicting the behaviour of PBT/vPvB substance concentrations and the variability of estimates of concentration and environmental load of a PBT and vPvB substance. This is combined with consideration of how those outputs can be transformed into values for decision/making in the context of environmental impact/cost assessment for a REACH authorisation SEA. Key variables that determine the sensitivity of the model are the substance half-life in the environmental compartment of interest, the animal toxicology of the substance, and the release rate of the substance. The influence of these variables on stock dynamics is considered using two separate example PBT and vPvB substances. In addition, we illustrate how the variability of parameters, even the absence of any reliable information, can affect the outcome and decision-making in the SEA. The conclusions indicate a strong need for clear guidance on understanding and presenting environmental concentrations and impacts, so that they can be used in the environmental decision-making of PBT/vPvB substances at the AFU and the NU scenarios. Such guidance should include a framework for assessment and presentation of PBT/vPvB residence time and stock dynamics.

TU354 The role of nutrition as a confounder for human cumulative risk assessment

M.C. Petriello, University of Kentucky / Toxicology; B.J. Newsome, University of Kentucky / Chemistry; B. Hennig, University of Kentucky / Superfund Research Center

Cumulative risk assessment is complex and may be influenced by chemical as well as non-chemical stressors or buffers. An emerging body of evidence correlates persistent environmental toxicants, such as dioxins and complex PCB mixtures, with human diseases like atherosclerosis. Many communities that reside around hazardous waste sites face daily pollutant exposure risks in addition to the impact of a multitude of other physiological and social stressors. One such confounding stress is sub-optimal nutrition, which has now been shown to exacerbate toxicant-induced disease. The scenario of individuals both at risk from a pollutant and chronically inflamed and oxidative stress is now synergistically poised to promote a more profound disease phenotype. In contrast to sub-optimal nutrition, evidence now shows that diets focused on healthful nutrition (e.g., diets centered on fruits, vegetables and fatty foods) may act as buffers and thus be able to counteract the detrimental effects of POPs such as PCB mixtures. We have shown that mice supplemented with diets that were fortified against PCB-induced inflammation and oxidative stress by exhibiting a more efficient antioxidant response. Although complete remediation of persistent environmental pollutants may be necessary to eliminate their contribution to disease pathologies, this process is extremely time-consuming and cost-prohibitive. Therefore, it is necessary to identify easily available modulators of toxicant-induced disease. Bioactive food components with antioxidant and/or anti-inflammatory properties, such as polyphenols and omega-3 polyunsaturated fatty acids, may act as a sensible means to provide physiological buffers against toxicant-related diseases. This new paradigm that the nutritional status of an individual can impact the toxicity of pollutants has important ramifications for cumulative risk assessment.

Understanding the nutritional makeup and tendencies of a community will allow scientists to better measure and estimate true risk of a specific pollutant or mixture. Finally, understanding the interplay between toxicology and nutrition, especially concerning complex mixtures, requires the development of more complex statistical analyses and models that will allow scientists to create personalized risk assessments for individually impacted human populations.

Risk assessment of chemical mixtures: trends, developments and bottlenecks for integrating exposure and effects (P)

TU355 Assessing exposure and risks levels of Biocidal Products: needs for Tier 1 screening tool and current issues

C. Durou, CEHTRA SARL; A. Barret, CEHTRA SAS; E. Beltran, CEHTRA SARL; L. Pontal, CEHTRA SAS; s. kirkham, CEHTRA UK ltd

The Biocidal Products Regulation (528/2012) has applied from 1 September 2013. The regulation requires an environmental risk assessment (ERA) to be performed on the active substance (AS) present in any biocidal product (BP) to be marketed on the EU market. Besides this provision for the AS, additional risk assessment, to the same standard as for the AS, shall be carried out for the precursor(s) of in-situ generated AS and for any substance of concern (SoC). The results from these assessments shall be integrated to produce an overall risk assessment for the BP.

Finally, the assessment shall take into account any cumulative or synergistic effects. Various tools have been made available to assess the exposure of chemicals for the environment. Among these tools, EUSES has been extensively used for the assessment of risks posed by AS under the review program of existing AS. The exposure assessment shall address the typical use claimed on the label alongside a realistic worst-case scenario. Generic product type exposure scenarios have been developed (RIVM/EUBEES Program/OECD) and some of these biocide uses are incorporated in EUSES 2.1.2 version (2012). However, some ESDs are not updated, are not included as they were developed after the last update of EUSES, or the EUSES ESD contains errors. As an example, for disinfectants used in food and feed areas (Product Type 4), a generic ESD is publicly available but has not been included in EUSES. For many biocide uses, no ESD have been developed and will require tailor-made scenarios to be performed. CEHTRA using Microsoft Excel® has developed a unique Tier 1 screening tool to allow for the comparison of environmental exposure and risk of multiple substances in a single BP. In addition, the tool is developed in flexible way to meet the needs for adaptation in case of uses of BP not covered by any generic ESD or new generic ESD. The purpose of this work is to initially review the issues with ERA for biocide products, the need for an adaptable assessment tool able to handle these complexities. An example PT 4 ERA will then be presented using the tool. To conclude, the poster will compare and contrast an assessment performed using the CEHTRA-Tier 1 tool against one performed using the current standard tools.

TU356 Toxicological assessment of intentional and unintentional mixtures within the European regulations

S. Van der Linden, Biodetection Systems BV; A. Kienzler, European Commission - Joint Research Centre / IHPC System Toxicology Unit - EURL ECVM; S. Bopp; A. Worth, European Commission Joint Research Centre / IHCP Systems Toxicology Unit and EURL ECVM; E. Berggren, European Commission Joint Research Centre (JRC) - Institute for Health and Consumer Protection - IC & EU Reference Laboratory for Alternatives to Animal Testing EURL ECVM

While humans and the environment are continuously exposed to a multitude of substances via different routes of exposure, current regulatory risk assessment mainly focuses on the assessment of individual substances via a single exposure route. However, adverse effects might arise from exposure to a single compound originating from several different sources (combined exposure), or from exposure to different compounds contributing jointly to the same adverse effect (mixture exposure). Due to the largely unknown exposure to individual compounds and potentially unlimited combinations thereof, the toxicological assessment of mixtures is extremely complex. Different types of mixtures are identified in current EU regulations, but currently no clear methodological approaches are defined on how to deal with the problem of mixture exposure. This gap in the EU regulatory assessment requirements has recently gained more attention, following a 2012 Commission Communication on the Combined Effects of Chemicals. Currently, research is undertaken to tackle the major problems in implementing a scientifically sound assessment of mixtures, which have to be based as far as possible on non-animal testing methods. Here, an overview of the current regulatory requirements for the assessment of mixtures is presented, covering the current state of the art in the evaluation of EUC and EU Reference Laboratory for Alternatives to Animal Testing EURL ECVM. How and to what extent mixtures are addressed in different legal frameworks is investigated and indications of discrepancies are given. The different scientific tools to assess mixtures are presented, including their current use and how they are (or can potentially) be applied in EU legislation. The most important factors hampering current legal implementation will be summarized as well as how these factors can be taken into account in the currently proposed frameworks on the toxicological assessment of mixtures.
Relevance of EDC mixture to current environmental relevant exposure. Even though most chemicals regulation is still conducted on a chemical by chemical basis, mixture toxicity is achieving increasing attention. The scientific understanding has increased substantially in the last decades, and a general consensus now seems to have been aced that concentration addition is a suitable model for default estimations of mixture effects. One of the major challenges is therefore how to select specific chemicals for actual mixture toxicity assessments. Persistent chemicals are likely to be present in the environment for an extended period of time, thus increasing the likelihood of them being present in environmentally found mixtures. Persistent, bioaccumulative and toxic (PBT) chemicals are therefore a highly relevant group of chemicals to consider for mixture toxicity regulation. The present study evaluates to what extent a number of PBT-like chemicals possess concern beyond that of the individual components. Firstly, the effects of three chemicals with PBT-like properties (acetyl cedrene, pyrene and triclosan) was examined on the freshwater snail, Potamogeton antipodorum. Secondly, mixture bioaccumulation of the same three chemicals were assessed experimentally. Thirdly, a review of the existing literature on mixture effects of 132 PBT-like chemicals were conducted to evaluate the quantity and quality of existing data and to indicate further research required within the field. The experimental data illustrated that no observed effect concentrations (NOECs) of PBT-like chemicals can lead to effects when mixed. Such “something from nothing” effects will be underestimated if mixture effects are not considered for environmental risk assessment. The review illustrated that the scientific knowledge of PBT-like mixtures is still limited, thus demonstrating a need for future focus on such mixtures.

TU358 Can we apply concentration additive and independent action mixture effect model to species sensitivity distribution? - An experimental validation using herbicides

T. Nagai, K. Taya, I. Kitayama, National Institute for Agro-Environmental Sciences

Conventional prediction models of chemical mixture, concentration additive (CA) and independent action (IA), have been used for mixture effect assessment. The applications of CA and IA to single species toxicity have a lot of examples of experimental validation. The calculations of sensitivity distribution (SSD) have also been used to calculate multi-substance potentially affected fraction (msPAF) for ecological risk assessment of chemical mixture. However, msPAF calculation has not been validated experimentally. Therefore, we tried to validate the application as a case study using herbicides which are toxic to algae. We tested the mixture toxicity of herbicides on the growth of five periphytic algae, chlorella. In order to guide future research within the field. The experimental data illustrated that no observed effect concentrations (NOECs) of PBT-like chemicals can lead to effects when mixed. Such “something from nothing” effects will be underestimated if mixture effects are not considered for environmental risk assessment. The review illustrated that the scientific knowledge of PBT-like mixtures is still limited, thus demonstrating a need for future focus on such mixtures.

TU359 Relevance of EDC mixture effects for ecological risk assessment - a case study of propiconazole and resorcinol with L. stagnalis as a model organism

A.S. Andersen, ENSPAC; T. Lee, Roskilde University; A. Palmqvist, K. Syberg, Roskilde University / Department of Environmental Social and Spatial Change; K. Jensen, Roskilde University

There is growing evidence that a number of chemicals may possess endocrine disrupting activity, and that these effects can occur at concentrations that are similar to current environmental relevant exposure levels. There are concerns that decline in wildlife species and populations might be linked to exposure of endocrine disrupting chemicals (EDC’s). When species and populations decline it may influence the ecological context by changing the composition of the biological community and the ecosystem function and services which we depend on. Traditional regulation of chemicals in EU is based on risk assessments of the individual chemicals. Since chemicals end up in mixtures in the environment the effects of these mixtures need to be accounted for, if we want to realistically and safely estimate risk for the environment. In the current study the potential EDC’s propiconazole and resorcinol have been tested on the freshwater gastropod Lymnaea stagnalis. Partial life cycle tests, with the endpoints fecundity, egg quality, timing and survival, were performed for the two compounds individually and in mixture to explore whether they have synergistic, additive or antagonistic effects. Based on the results from this case-study the EU regulation concerning mixtures and EDC’s in the environment is discussed.

TU360 An online database on the ecotoxicity of pesticide and pharmaceutical binary mixtures

J. Kim, R. Pasupuleti, B.E. Lee, M. Fischer, KIST Europe / Environment and Bio Group

The interest and studies on mixture toxicity among chemicals are continuously increasing in ecotoxicology because the mixture toxicity can be provoked by combined effects of two or more substances even at their no-observed-effect concentrations in the environment. Various mixture toxicity datasets can contribute not only to provide information of potential hazard of mixtures to risk assessors, but also to the development of models for developing prediction models. However, there is a lack of systematic databases on the ecotoxicity of mixtures which efficiently provide useful information on combinations of substances, toxicity targets, organisms, toxicity endpoints, literature cited, etc. Therefore, the objective of this study was to develop an online database on the ecotoxicity of mixtures with focusing on pesticide and pharmaceutical binary mixtures.

TU361 Prediction of synergistic toxicity of mixtures to Vibrio fischeri using bioinformatic and chemoinformatic techniques

M. Fischer, J. Kim, KIST Europe / Environment and Bio Group; V. Helm, University of Saarland / Center for Bioinformatics

The interest and studies on mixture toxicity among chemicals are continuously increasing in ecotoxicology because the mixture toxicity can be provoked by combined effects of two or more substances even at their no-observed-effect concentrations. The combined effects can be divided into additive, synergism, antagonism, and potentiation. Conventionally, concentration (CA) and independent action (IA) models have been frequently employed to estimate the additive toxicity. Integrated CA and IA models have been also developed to overcome the limitations of the conventional models. However, there is a lack of synergism models to predict the synergistic toxicity of mixtures. Therefore, the objective of this study was to develop an approach to predict the synergistic toxicity of mixtures to Vibrio fischeri, a luminous bacterium, using different bio- and chemo-informatic techniques, e.g. calculations on structural similarity, chemical-protein and protein-protein interaction networks. This study highlights that the bio- and chemo-informatics based approach showed a potential to be used for predicting the synergistic toxicity. Further studies are required to evaluate this method with different organisms and target mixtures.

TU362 A web-based estimation tool for classification, labelling and packaging of mixtures

J. Kim, B.E. Lee, KIST Europe / Environment and Bio Group; S. Kim, KIST Europe / Chemical Management Lab

From 1 January 2015, chemical industries are required to classify and label mixtures according to the European regulation on classification, labelling and packaging of substances and mixtures (CLP). The CLP regulation entered into force on 20 January 2009 so that existing European system on hazard classification and labelling of chemical products, e.g. Directives 67/548/EEC and 1999/45/EC, could also be applied. Furthermore, under the CLP, the classification of mixtures can be estimated on the basis of hazard information of mixture itself, similar mixtures, or components in a mixture. Component-based estimation approaches appear to be practically used for the CLP estimation, due to the lack of toxicity test data on various mixtures and chemical combinations. Accurate and timely CLP estimation is important; however, technical guidance documents and CLP estimation algorithms for mixtures are extremely complicated and require expert judgement. This seems to be a burden especially to small and medium-sized enterprises. Therefore, in this study, a web-based CLP estimation tool for mixtures was developed so that it could support chemical industries to effectively comply with the CLP regulation.

TU363 USEPA’s Dermal Slope Factor for Benzo(a)pyrene Predicts That Skin Cancer in London is Caused by PAHs in Soil

B.H. Magee, ARCADIS; N. Forsberg, ARCADIS US Inc / IEST; K. Baker, ARCADIS
USEPA’s draft toxicological assessment of Benzo(a)pyrene (BaP) was released in September 2014. The proposed Dermal Slope Factor (DSF) of 0.006 (µg/d)1. USEPA’s proposed DSF derived from mouse skin studies is true, it would predict that a large fraction of non-melanoma skin cancers in the is caused by low level dermal exposure to BaP and other PAHs that have BaP relative potency factors from soils, grilled foods, coal tar containing consumer products, and tobacco. USEPA’s proposed DSF, a screening level population risk assessment was performed on urban soils London. Vane et al. (2014) recently tested 76 surface soil samples from London for PAHs. The mean benzo(a)pyrene toxic equivalence (µg-TE) using the EPA (2010) proposed Relative Potency Factors is 6.2 mg/kg. Emboö-Mattingly, et al. (2014) have analyzed a variety of PAH mixtures with a method that includes the 16 additional proposed potentially carcinogenic PAHs. They found that low level exposure allows an assessment more consistent with the observed ecological status than the traditional individual or additive methods.

TU366 What are relevant compounds from an effect perspective? - Mode of action considerations for compounds and mixtures detected in different European river systems. D. Zwart, RIVM / Centre for Sustainability Environment and Health; S. Schmidt, N. Ost, Helmholtz centre for environmental research - UFZ; T. Schulze, M. Krauss, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; K. Tollefsen, NIVA / Ecotoxicology and Risk Assessment; R. Altenburger, UFZ Centre for Environmental Research / Department of Bioanalytical Ecotoxicology

Environmental risk assessment for chemicals is still based on a few regulated compounds. Nowadays, chemists are able to analyze and detect thousands of compounds even in very low concentrations. Neither the biological effects of single anthropogenic compounds in the environment nor the potential adversity of combined effects of chemicals concentration combinations and action mechanisms and risk evaluation, so far. Chemicals can be grouped in different ways, e.g. according to their chemical structural properties (chemical group), their application domains (use group), their frequencies of occurrence or environmental concentrations, but also with respect to their biological modes of action. It is well known, that e.g. Organophosphates act as Acetylcholine Esterase inhibitors. Linking such mechanistic information with measured environmental concentrations might improve the assessment of combined effects. Therefore, we performed a literature and database research on the modes of action of about 1000 compounds that have been detected in different European river catchments. In a second step we filtered the data for compounds potentially relevant for ecotoxicity by a very rough hazard estimation using the ratios of the detected concentrations and measured (in vivo) or predicted effect concentrations. We analyzed whether certain modes of action are potentially predominant and how the picture is changing when similar acting compounds are considered as a group. The poster will summarize the findings of this exercise and give an outlook on how the knowledge of modes of action might be further implemented in assay development and/or compound selection on the way towards a biological effect assessment of chemical mixtures.

TU367 Initial risk assessment and effects of mixtures of plant protection products K. Petersen, Norwegian Institute for Water Research; M. Stenrod, Bioforsk; T. Backhaus, University of Gothenburg / Department of Biological and Environmental Sciences / Natural and Agricultural Science; K. Tollefsen, NIVA / Ecotoxicology and Risk Assessment, From a study conducted in 2013, it was found that the measured concentrations of co-occurring plant protection products (PPPs) in selected agricultural streams in Norway might pose an environmental risk based on an initial cumulative risk assessment. The scenarios having the highest predicted environmental risk were chosen for a follow up study including laboratory tests and chemical analysis to validate the approach for cumulative risk assessment and to evaluate the use of assessment factors. A synthetic mixture based on the measured concentrations of detected PPPs in a stream with one of the highest predicted environmental risks was prepared. The ratio between the compounds in the synthetic mixture was made to reflect the ratio detected in the environment and was kept constant over all mixture concentrations. A concentration of the mixture of the synthetic mixture concentrations, was tested on Chlamydosorus reinhardtii, daphnia magna and lenna minor to investigate potential effects as indicated by the predicted environmental risk. Chemical analysis was performed on the highest and lowest mixture concentration in addition to controls. Dose-response curves were developed, making it possible to evaluate at which mixture concentrations effects were first observed for the two different species. In order to study potential synergistic effects of PPPs, a second stream was chosen for the follow up study. No compounds with known synergistic effect were detected in concentrations above limit of quantification in the stream, but two compounds with known synergistic effect were reported to be much used in the area. Therefore it was of interest to include these two compounds in a fractionated factorial design together with the synthetic mixture based on the environmental concentrations in the stream. The different mixture combinations of the synthetic mixture and the two additional compounds were tested on daphnia magna. The initial predictive risk assessment was improved by filling data gaps for missing effect data. A comparison of ad hoc derived PNECs, derived EQSs from the literature and environmental hazard values
was performed to identify if the difference in the values between these approaches was due to use of different effect data or by different use of assessment factors. The results are believed to contribute to the validation of the cumulative predictive risk assessment approach and to evaluate the use of assessment factors in this approach.

Science communication - Concepts and activities from past and current research projects (P)

TU368
A conceptual framework for solutions-oriented prioritization and management of chemical pollutants
J. Munkit, IVL Swedish Environmental Research Institute Ltd; E. Borgstrom-Lundén, IVL Swedish Environmental Research Institute; M. Rahmberg, T.V. Rydberg, IVL Swedish Environmental Research Institute; W. Brack, Helmholzt Centre for Environmental Research UFZ / Effect Directed Analysis; A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health; G. Engelen, Vlaamse Instelling voor Technologisch Onderzoek VITO / E. van E. Stroobants, Flemish Community / E. van E. Stroobants, Flemish Community / e.V. / Sustainable Products Material Flows Division; J. van Gils, DELTARES; J. Slobodnik, Environmental Institute; R. Altenburger, UFC Centre for Environmental Research / Department of Bioanalytical Ecotoxicology; L. Posthuma, RIVM / Centre for Sustainability Environment and Health

SOLUTIONS is a project based on a solutions-oriented approach. This approach includes the development of a logical framework and guidance on how analytical tools and models can be applied to provide answers to specific questions and to achieve results which form the basis for risk assessment and decision-making. The solutions-oriented approach requires that management options and stakeholder and regulatory views and problems should be addressed already at the problem-formation stage and not only after completing the risk assessment and prioritisation. One of the primary tasks in the project is to develop a conceptual framework for this solutions-oriented approach for surface water contamination.

The conceptual framework for this solutions-oriented approach will clearly demonstrate: tools, models and key expertise required; the requirements for accuracy, validation and application scales; the sequence of application; and abatement measures. Its early design and its application should improve the utility of the risk assessment. Also outside a research lab, social, cultural and economic means of risk are inseparably present in decisions made under uncertainty. Risk communication clearly requires interdisciplinary approaches. Interdisciplinarity, especially in the area of climate change and environmental hazards, is gaining in popularity. Still research teams that cross several disciplines face many challenges at the institutional level, such as limited access to funding as well as communication obstacles. The aim of our study is twofold. First, we identified benefits and potential challenges to interdisciplinary work in the area of environmental risk communication. Second, we compared several written samples related to the watch list substances EE2, E2 and E1. For this purpose, 20 surface water and 20 wastewater samples across Europe will be collected and analysed. Approximately 20 institutes or agencies from 13 nations will be involved in the project. Detection methods covered: Best possible chemical analysis (Joint Research Centre (JRC), IT, and Federal Institute of Hydrology (BfG), DE) and effect-based methods relating the effect-based monitoring to compounds currently going through the OECD validation process or being developed as ISO standards. Results and discussion: We mainly discuss project characteristics and objectives in this abstract: a) Promoting reliable screening methods to support the monitoring of endocrine disrupting activity in wastewater and surface water b) Harmonising monitoring options across Europe c) Linking reliable effect-based approaches and effect-directed analysis for estrogen monitoring d) Supporting national and EU monitoring for endocrine disruptors

Conclusions and expected outcomes: Only a limited number of institutes in Europe currently have the capacity to quantify the steroidal estrogens EE2 and E2 at their suggested EQS levels. This could cause problems for the EU watch list mechanism, where reliable exposure data are required. In this project we will harmonise monitoring methods and develop a joint harmonised “watch list” for estrogen monitoring. The project will focus on specific effect-based methods and best possible analytical methods related to the current monitoring difficulties by measuring the estrogenic activity of environmental samples in a cost-efficient way. Project description: The project will focus on specific effect-based methods and best possible analytical methods related to the current monitoring difficulties by measuring the estrogenic activity of environmental samples in a cost-efficient way.

TU369
Interdisciplinary collaborations and risk communication
A. Hunka, University of Twente / Faculty of Behavioural Sciences; M. Meli, Rifcon GmbH; A. Palmqvist, Roskilde University / Department of Environmental Sociology; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; J. Munthe, RIVM / Centre for Sustainability Environment and Health

Environmental risk communication draws upon environmental and human toxicology concerns, utilises methods of social science, natural science and media studies and impacts the area of public perceptions, understandings of science and risk management. Also outside a research lab, social, cultural and economic means of risk are inseparably present in decisions made under uncertainty. Risk communication clearly requires interdisciplinary approaches. Interdisciplinarity, especially in the area of climate change and environmental hazards, is gaining in popularity. Still research teams that cross several disciplines face many challenges at the institutional level, such as limited access to funding as well as communication obstacles. The aim of our study is twofold. First, we identified benefits and potential challenges to interdisciplinary work in the area of environmental risk communication. Second, we compared several written samples related to risk communication (scientific papers, popular science, outreach material) written by experts in different fields: sociology, ecology, media studies, political science, chemistry and by interdisciplinary teams, in order to identify whether interdisciplinary collaborations affect the communication about risk. We chose a single topic of pesticide risk, as it tends to generate interest among scientists from different disciplines. It is also widely covered by the popular press, which can be used as a benchmark for risk communication accessibility, as the general public increasingly turns to the internet and other media in search of information about risks. Our preliminary findings show that different disciplines remain disparate, usually自主研发, and in some cases even if the studied topic is identical. Interdisciplinary studies tend to be popular in applied science, where the goal is to address a practical problem, rather than advance fundamental knowledge. Interdisciplinary teams, on the one hand, often produce more accessible output, since researchers with different background have to collaborate and understand each other. On the other hand, it is not uncommon that only one of the contributing discipline’s terminology dominates in such projects. We conclude that it is necessary to address institutional obstacles to interdisciplinary research and provide early training in interdisciplinary communication to promote interdisciplinary collaborations and, in turn, better risk communication.

TU370
Effect-based and chemical analytical monitoring for the steroidal estrogens: An international project to cope with a monitoring challenge
R. Kueh, Swiss Centre for Applied Ecotoxicology EAWAG - EPF; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology; H. Hollert, RWTH Aachen University / Department of Environmental Research; E. Vermeiren, Eawag / Dept of Environmental Toxicology; S. Buchinger, Federal Institute of Hydrology / Department G Biochemistry Ecotoxicology; P. Behnisch, BioDetection Systems; B. Jarosova, Masaryk University / Faculty of Science RecetoX; I. Lettieri, RN. N. Carvalho, European Commission - Joint Research Centre / Institute for Environment and Sustainability; R. Loos, European Commission DG Joint Research Centre / Institute for Environment and Sustainability; H. Clayton, Directorate General Environment and Sustainability; O. Perceval, ONEMA DAST; A. S. Ait-Aissa, INERIS / Ecotoxicology Unit; N. Crestot, INERIS; G. Reifischetter, Biochemistry and Ecotoxicology; T. Ternes, Bundesanstalt für Gewaesserkunde; C. Heiss, general aspects of water and soil protection; T. Seiler. RWTH Aachen University / Ecotoxicology and Toxicology; P.Y. Küster, K. Koehler, D. Duttweiler, C. Valsecchi, Water Research Institute - Italian National Research Council IRSA-CNR; m. carere, Regulatory and risk assessment background and needs: In the context of the European Union (EU) Water Framework Directive (WFD) it is recognised that pharmaceuticals may pose a risk to the quality of European water bodies. Three substances with pharmaceutical use are included in the first so-called “watch list.” However, despite harmonizing of methodological concerns, it is difficult to meet the detection limits of most existing routine analytical methods and the high cost of high-end analytical methods. Sensitive effect-based methods are of reducing the current monitoring difficulties by measuring the estrogenic activity of environmental samples in a cost-efficient way.

Project description: The project will focus on specific effect-based methods and best possible analytical methods related to the current monitoring difficulties by measuring the estrogenic activity of environmental samples in a cost-efficient way. Project description: The project will focus on specific effect-based methods and best possible analytical methods related to the current monitoring difficulties by measuring the estrogenic activity of environmental samples in a cost-efficient way.
workshop was organised on 10-12 September 2014 at the Sydney Institute of Marine Sciences, Sydney, Australia. This workshop aimed to explore the potential offered by these new approaches to characterise stressor regimes, to explore stressor-response relationships among biota, to design better early-warning systems and to develop smart tools to support sustainable management of human activities through more efficient regulation. This presentation will discuss the limitations of the current assessment approaches and how multiple stressors at large scales can be better predicted in ecological risk assessments to inform the development of more efficient and preventive management policies based on adaptive management in the future. At the same time, a future risk assessment paradigm that overcomes these limitations will be presented. It is envisaged that through greater integration of approaches among different scientific disciplines, together with the application of new and emerging tools such as ‘Big Data’, ecological modelling and incorporation of ecosystem services endpoints will make this achievable.

TU372 DERIVING ENVIRONMENTAL QUALITY STANDARDS FOR PERFLUOROOCACETIC ACID (PFOA) AND RELATED SHORT CHAIN PERFLUOROBUTANOIC ACIDS
S. Valsecchi, Water Research Institute - Italian National Research Council
IRS-CNR: S. Polesello, Water Research Institute-CNR / Water Research Institute; S. Capri, Water Research Institute - National Research Council; E. Prevosti, IRS-CNR Water Research Institute; m. carere; L. Lucentini, E. Ferretti, R. Crebelli, ISSI Istituto Superiore di Sanità; D. Conti, S. Balzano, Istituto Superiore per la Protezione e la Ricerca Ambientale ISPRA; M.G. Simeone, ISPRA- Instituto for Environmental Protection and Research; F. Aste, MATTM Ministry of the Environment Land and Sea

The evidence that in Northern Italy significant sources of perfluoroalkylacids (PFAA) are present induced the Italian government to establish a Working Group on Environmental Quality Standard (EQS) for PFAA in order to include some of them in the list of national environmental risk (Ministerial Decree 26/02/2010) for surface water monitoring and classification in the context of the Water Framework Directive (2000/60/EC). The substances have been chosen by considering the statistical distribution of concentrations and frequency of detection in the Italian surface and ground waters. The substances in the list included perfluorooctanoic acid (PFOA) and related short chain (number of carbon < 7) perfluorocarboxylic acids such as perfluorobutanolic (PFBA), perfluoropentanoic (PFPeA) and perfluorohexanoic acid (PFHxA). Perfluorobutanesulfonic acid (PFBS), which is a common substitute of perfluorooctanesulfonic acid (PFOS), has been included too. For each of these substances a dossier has been prepared which collects available data on regulation, physico-chemical properties, emission and sources, occurrence, acute and chronic toxicity on aquatic species and mammals, including humans. Quality standards (QS) have been derived for the different protection objectives (pelagic and benthic communities, predators by secondary poisoning, human health via consumption of fishery products and water) according to the protocol proposed by the Technical Guidance Document on deriving EQS (CIS-WFD Guidance n. 27). The most protective QS has been chosen as the national EQS. For all compounds it is not possible to derive a QS for sediments because in some cases compounds do not accumulate in sediment and in other cases data on toxicity on benthic community are lacking. For PFBA, PFPeA, PFHxA and PFBS it is not possible also to back-calculate a QS for water from QS for biota because they have low bioaccumulation properties or data on biocaccumulation factors (BCF) are lacking. For these compounds limits derived for the protection of human health by drinking water are used as EQS. For PFOA it is possible to derive a QS for biota both for the protection of predators and human via fish consumption and back-calculate QS for water. The calculation resulted in a QS for secondary poisoning of 0.1 µg L\(^{-1}\) which has been proposed as EQS for PFOA in internal surface waters.

TU373 Assayment of various contaminants present in sediments and water in Khalid Khor, Sharjah, United Arab Emirates
F. Samara, B. Soghomonian, S.L. Krutenson, American University of Sharjah / Biology Chemistry and Environmental Sciences

Over the last 20 years the United Arab Emirates (UAE) has been going through several changes and improvements which have led to rapid urbanization of the country especially in cities such as Dubai, Abu Dhabi and Sharjah. Samples were collected from Khalid Khor port and Khalid Khor Lake to assess the water quality. The water quality measurements were taken on site by using various meters. The Electronical analysis for water and Sediment samples were performed using the Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES) and organic analysis was conducted using Gas Chromatograph- Mass Spectroscopy (GC-MS). Various parameters such as pH and alkalinity indicated normal water quality levels. On the other hand, bacterial contamination was detected in the port ranging between 300-10140 organisms/100ml. The concentration of heavy and trace metals in sediments ranged from 0.066- 12668 mg/Kg, indicating low to average inorganic contamination. Organic compounds such as Benzene, Benz[a]anthracene, Benzo[fluoranthene and O-Xylene were qualitatively detected in the sediments, thus, indicating that Khalid Khor has some contamination that could potentially affect the health impacts to residents in Sharjah.

TU376 Filter-feeding polychaete Sabella spallanzanii as biomediator of aquatic wastes
I. Granada, Escola Superior de Turismo e Tecnologia do Mar - Instituto Politécnico
Aquaculture practices, like most other human activities affect the environment in different ways. Recently, some attention has been dedicated to the effects of discharges of effluents from certain types of aquaculture. Aquaculture operations cause the release of metabolic waste products which is considered one of the most important factors causing organic and inorganic loading in the vicinities of aquatic farms. Suspended solids have been considered one of the most important waste products affecting the quality of the receiving waters and their environment. Also, both in aquaculture facilities and in natural aquatic environment, the occurrence of several diseases have emerged as a serious economic and ecological issue, and are a significant constraint to the expansion of the industry. The control of endemic disease prevention, on-year costs, is one of the main concerns. Integrated Multi-trophic Aquaculture (IMTA) has been proposed to achieve environmental sustainability through biomass of aquaculture wastes which has advantages that may include economic stability by product diversification and risk reduction, and social acceptability through best management practices. IMTA is a practice in which the by-products from one species are recycled to become inputs for another through the cultivation, in the right proportions, of fed aquaculture species (e.g. fish/shrimp/.../p) with organic extractive species (e.g. suspension and deposit feeders), and inorganic extractive aquaculture species (e.g. seaweeds). The reduction of microbial pollution within aquaculture can be achieved by the use of living organisms. Filter-feeding marine macroinvertebrates filter large volumes of water for their food requirements and exert high efficiency in retaining small particles including bacteria. Some authors recover these back by a magnetic trap and to reuse the calcium carbonate in subsequent batches without additional costs. In this way, fouling is confined and guarantees COD values in the purified permeate stream of about 1.3 g L⁻¹. This value complies with irrigation water quality standards. Moreover, the adoption of this treatment sequence leads to a reduction of the required membrane area, equal to 104.6 m² and 81.4 m² for the UF and NF membranes, respectively. As a consequence, a limited overdesign of the membrane plant is needed. Additionally to this, the longevity of the membranes sensibly increases, reducing the capital and operating costs of the treatment, which results to be economically feasible.

**Keywords:** olive mill wastewater, membranes, nanofiltration, advanced oxidation.

**References:**

**TU379**

**Photo-Fenton treatment eliminates drugs of abuse pollution in natural waters and reduces toxicity**

**M. Catala Rodriguez, N. Domínguez, A. Migens, Universidad Rey Juan Carlos / Biology and Geology Physics and Inorganic Chemistry; R. Molina, F. Martínez, Universidad Rey Juan Carlos / Energy Chemical Environmental Mechanical Technology and Analytical Chemistry; Y. Valcárcel, Universidad Rey Juan Carlos / Department of PreventiveMedicine Public Health Immunology and MedicalMicrobiology; N. Mastroianni, Institute for Environmental Assessment and Water Research / Environmental Chemistry; M. López de Alda, Institute of Environmental Assessment and Water Research IDAE-CSIC / Environmental Chemistry; D. Barceló, IQAB-CSIC / Institute for Environmental Assessment and Water Research; Y. Segura, Universidad Rey Juan Carlos / Energy Chemical Environmental Mechanical Technology and Analytical Chemistry**

This paper investigates the elimination of drugs of abuse 25 from six different chemical classes/and their metabolites in natural fluorial waters (nearby the output of the sewage system) byMineralization of these substances and toxicity. 

**Abstract:**
Photo-Fenton process has been tested. However, toxicological analysis demonstrated that/nures/0.6 g/L of the Fe catalyst allowed a complete elimination of acute and chronic/toxicity measured as inhibition of fern spore mitochondrial activity, lower catalyst loadings/unre/ efficient for toxicity elimination. These results evidence that the combination of a toxicological tests with chemical analysis is mandatory in order to establish the harmfulness of the waste treatment technologies based on advanced oxidation processes.

**Keywords:**
Optimization of the azo dye Disperse Yellow 3 oxidation by heat-activated persulfate using Box-Behnken design
J.E. Silveira, Ingenieria Quimica; X. Xu, G. Pliego, J. Zazo, J. Casas, Universidad Autónoma de Madrid

Response surface methodology (RSM) based on Box-Behnken (BBD) design was successfully applied to the optimization in the operating conditions of the oxidation of the azo dye Disperse Yellow 3 (DY3). All experiments were performed in the glass water-jacketed and the temperature was maintained by circulating the water through the jacket around the reactor. The initial DY3 concentration of 70 mg L⁻¹ was used. It was employed the factorial design with 3 independent variables: SO₄²⁻ (mM), temperature (°C) and reaction time (min) upon the Total Organic Carbon (TOC) and absorbance removal were evaluated through the jacket around the reactor. The inicial DY3 concentration of 70 mg L⁻¹ was used. It was employed the factorial design with 3 independent variables: SO₄²⁻ (mM), temperature (°C) and reaction time (min) upon the Total Organic Carbon (TOC) and absorbance removal were evaluated.

**Keywords:**
 response surface methodology (RSM), Disperse Yellow 3, sulfate radical, toxicity, Vibrio fischeri.

**TU381**

**Integrating biomedical intervention strategies with novel pollutant remediation platforms to address POP risk reduction**

B.J. Newsome, University of Kentucky / Chemistry; B. Hennig, University of
Kentucky / Superfund Research Center

Due to their relative chemical stability and ubiquity in the environment, chlorinated organic contaminants such as polychlorinated biphenyls (PCBs) pose significant health risks and enduring remediation challenges. Addressing associated needs, though, relies on both effectively removing pollutants from contaminated sites as well as modulating toxicity induced by existing pollutant body burden. This work highlights a sustainable approach to addressing a pervasive global health concern, entrar with methods that support intervention, remediation and counterdevelopment for pollutant removal and remediation. A polyphenol-functionalized, nanocomposite system has been developed which combines the biomimetic binding capabilities of nutrient polyphenols with the separation and heating capabilities of superparamagnetic iron oxide NPs for the capture/sensing of organic contaminants in polluted water sources. Magnetic nanocomposite microparticles (MMNs) incorporating the fluorescent polyphenols quer cetin and curcumin exhibit high affinity for model organic pollutants followed by rapid magnetic separation, addressing the need for sustainable pollutant remediation. Further, a membrane-based, redox-mediated system for organic pollutant dechlorination has been developed to work in tandem with the pollutant capture/removal system in an attempt to more complete and environmentally conscious remediation. Further work has been performed to both better understand health concerns associated with environmental toxicants such as PCBs and to determine effective methods for modulating their toxicity. This research has shown that PCB remediation through dechlorination is a viable technique for decreasing endotheial inflammation, although complete dechlorination to biphenyl is necessary to effectively eliminate superoxide production. NF-κB activation is a key feature of the volutology. To define the ideal polyphenol for in vivo PCB toxicity by up-regulating a battery of antioxidant enzymes transcriptionally controlled by AhR and Nrf2 proteins. This work combined with research translation efforts to inform regulators, transfer remediation technologies, and engage trainees in communication to diverse stakeholders, this approach serves as a model for increasing researcher and stakeholder environmental health literacy as we seek to more effectively address needs of affected populations.

TU382

In situ biological treatment of nitrate-polluted groundwater for drinking water production
J. Juhany, Fundacio CTM Centre Tecnologic / Environmental Technology; M. Calderer, Fundacio CTM Centre Tecnologic / Environment Technology Area; E. Vilanova, J. Font-Capo, J. Moliner, Ampho21 Consulting S.L.; R. Grau, E. Pinto, Catalana de Perforacions; M. Rovira, Fundacio CTM Centre Tecnologic

The study was conducted within the framework of Life+ InSiTrate project which is developing an in-situ treatment technology for drinking water production from nitrate-polluted groundwater with the aim to recover drinking water wells especially for small communities with a lack of other available freshwater sources. Initially, the most appropriate organic matter for denitrification was selected. The selection of the organic substrate is a crucial point when implementing biological treatments in nitrate-contaminated groundwater. The organic matter was found to serve as a model for increasing researcher and stakeholder environmental health literacy as we seek to more effectively address needs of affected populations.

TU383

Evaluation of the mutagenicity of the dye Acid Red 114 using HepG2 cells, before and after biodegradation process.
N. A. Corroque, Unesp - Institute of Biosciences / Biology; R. R. Hara, Unesp - UNIV ESTADUAL PAULISTA / Department of Biology; M. Hoshina, Unesp - Institute of Biology / Biology; M. A. Marin-Morales, UNIVERSIDADE ESTADUAL PAULISTA - UNESP / Department of Biology

Water bodies have been discharge targets of several industries, among them the textile industries. Among the most widely used textile dyes there are the azo dyes and within them the Acid Red 114. Biodegradation processes and biological adsorption have shown to be very promising to remove the colour and toxic effects of the dyes. This study aimed to evaluate the genotoxic and mutagenic potential of the dye Acid Red 114, by the micronucleus test in HepG2 cells, before and after biodegradation process by microorganisms isolated from the sludge of a textile industry. To assess the genotoxicity of the cells, they were exposed for 3 hours to the dye Acid Red 114 (concentrations of 50 μg/mL; 25 μg/mL; 2.5 μg/mL; 0.25 μg/mL and 0.025 μg/mL). The effects of dye were evaluated before and after the biodegradation process. The negative control – NC and positive control – PC were carried out, respectively with culture medium - MEM and PBS and culture medium - MEM and MMS. Two biodegradation processes were tested: biodegraded 1 (B1), performed with inoculum of Achromobacter denitrificans and biodegraded 2 (B2), performed with inoculum of Achromobacter denitrificans. The significance analysis was done by the statistical test ANOVA: one way/Dunnnett (p < 0.05). For the assays performed before the biodegradation, only the concentrations of 25 μg/mL and 0.25 μg/mL were not mutagenic for the HepG2 cells. After the biodegradation process, the concentrations tested were still mutagenic, indicating that the biodegradation by the biodegradation did not reduce the mutagenicity of the dye. However, the concentrations tested in B2 were not mutagenic, showing that the degrading bacterium was effective to eliminate the dye toxicity. Regarding the nuclear abnormalities (NA), the majority of the concentrations were genotoxic before the biodegradation but after biodegradation (B1 and B2), they did not present genotoxic activities. According to these data, it is possible to conclude that the method proposed by the biodegradation process was partially genotoxic and mutagenic. The B1 biodegradation process was partially effective in the removal of the dye toxicity, while B2 was totally effective in this removal.

TU384

Evaluation of heavy metal effect on phenol oxidation by manganese dioxide.J. Jiang, College of Environmental Science and Engineering / College of Environmental Science and Engineering; G. Sheng, Tongji University

As synthetic manganese oxides are effective for heavy metal adsorption and organic contaminant oxidation, their performance as decontamination agents for combined heavy metal and organic systems has not been fully evaluated. This study determined the adsorption of Co, Ni, Cu, Zn, Cd and Mn ions on synthetic MnO2, and its subsequent effect on the phenol oxidation. Single- and binary-metal adsorption experiments showed that the adsorption of these metals on MnO2 followed the order of Cu2+ > Co2+ > Ni2+ > Mn2+ > Cd2+ > Zn2+. The adsorptive selectivity increased monotonically with the adsorption quantity of the metals, except that Mn2+ which had generated by the biodegradation did not reduce the mutagenicity of the dye. However, the concentrations tested in B2 were not mutagenic, showing that the degrading bacterium was effective to eliminate the dye toxicity. Regarding the nuclear abnormalities (NA), the majority of the concentrations were genotoxic before the biodegradation but after biodegradation (B1 and B2), they did not present genotoxic activities. According to these data, it is possible to conclude that the method proposed by the biodegradation process was partially genotoxic and mutagenic. The B1 biodegradation process was partially effective in the removal of the dye toxicity, while B2 was totally effective in this removal.

TU385

Electroflootation applied in the treatment of wastewaters containing chlorimuron-ethyl, a sulfonylurea herbicide
R. R. Rachide Nunes, Universidade de Sao Paulo / Instituto de Quimica de Sao Carlos - IQSC; G. M. Morão, USP - Universidade de Sao Paulo / IQSC - Instituto de Quimica de Sao Carlos; M. D. Rezende, IQSC-USP

The electroflootation technique is commonly used for the separation of substances suspended in an aqueous phase. However, these studies are limited to the separation of wastes or contaminants, without destroying its contaminants. In this study, EF was applied as an advanced oxidation process, aiming to decontaminate wastewater containing sulfonylurea herbicides (chlorimuron-ethyl). Electrofloations were performed in an electrofloator reactor developed at the Laboratory of Advanced Chemical Engineering, Institute of Chemistry of Sao Carlos - University of Sao Paulo, Brazil. The reactor was built in acrylic and was endowed of aluminum electrodes. In addition, the anion electrode also was an agitation bar, that ensured the homogeneity of the solution during the process. Four assays were performed with varying salt concentrations (electrolyte - NaCl) and the direct electric current (I) applied on the electrodes. Chemical monitoring was performed to determine the efficiency of the electrochemical process. Analysis of water samples by HPLC allowed the electrochemical reaction to be monitored, indicating a disturbance of the reaction medium (presence of byproducts of degradation). Gas chromatography coupled with mass spectrometry (GC/MS) (Shimadzu GC-2010 MS QP2010 Plus model, Kyoto, Japan) was used to elucidate.
a mechanism of degradation and assisted in the identification of the byproducts. According to the chromatograms obtained by HPLC/UV, EF process changed the reaction medium, resulting in a displacement of the herbicide chromatographic peak and the appearance of bands without defined structure, related to generated byproducts. Based on the mass spectrum obtained, a mechanism of degradation was proposed, showing that EF does not totally destroy the chlorimuron-ethyl molecule, but it is successful in breaking important chemical bonds, responsible for the herbicide stability. This study provides important information about the possibility of promoting the oxidation of toxic organic compounds by electrofloation, generating less toxic and environmentally adjusted effluents.

TU386 Effect of resin dosage on sodium and chloride removal from olive mill wastewater by means of ion exchange
M. Víctor-Ortega, Chemical Engineering; J. Ochoando-Pulido, University of Granada / Chemical Engineering; A. Martinez-Ferez, University of Granada
Sodium and chloride ions are responsible for the high salinity of olive mill wastewater from an olive mill working with the two-phase decantation technology exiting a primary-secondary treatment (OMW-2ST) [1]. In this research work, the removal of sodium and chloride ions as a function of the resin dosage by Dowex Marathon C (strong acid cation exchange resin) and Amberlite IRA-67 (weak base anion exchange resin), respectively, was investigated. In both cases, the resin dosage was varied from 10 g/L to 175 g/L. Ion exchange (IE) experiments were carried out for 4 hours (until equilibrium was reached) and at room temperature. The experimental results revealed that sodium and chloride ion removal efficiency increases up to the optimum dosage beyond which the removal efficiency has no change with the resin dosage. As expected, the equilibrium concentration decreases with increasing adsorbent doses for given initial sodium and chloride concentrations, because for a fixed initial solute concentration, increasing adsorbent doses provide greater surface area or adsorption sites [2]. In this sense, a minimum resin dosage is 100 g/L for complete removal of sodium ions by means of Dowex Marathon C (about 97 % removal efficiency), whereas 175 g/L resin dosage was needed to completely uptake chloride ions (around 95 % adsorption efficiency).


TU387 Breakthrough curves study for adsorption of iron on Dowex Marathon C from olive mill wastewater
M. Víctor-Ortega, Chemical Engineering; J. Ochoando-Pulido, University of Granada / Chemical Engineering; A. Martinez-Ferez, University of Granada
A polyester resin catalyst is used during the secondary treatment (phase mill wastewater treatment (OMW) by means of advanced chemical oxidation process based on Fenton reaction [1]. For this reason, final olive mill effluent presents traces of iron and thus a complementary treatment, such as ion exchange, is needed. Ion exchange involves the use of synthetic resins where a presaturant ion on the solid phase (in this case Fe (III) ) is exchanged for the unwanted ions in the water [2]. In the present research work, a synthetic adsorbent study was carried out to evaluate the selective adsorption of iron (Dowex Marathon C) as adsorbent in a fixed-bed column for the removal of iron from OMW after secondary treatment. Continuous experiments were performed by setting operating temperature, flow rate and resin amount at 298 K, 10 L/H and 350 g, respectively. After each operational cycle, the resin was washed with diluted HCl. Then, the hydrogen form of the resin was washed with double distilled water to remove all the excess of acid. The dynamics of the adsorption process was modeled using the Adams-Bohart, Thomas, and Clark models. The models were used to predict the breakthrough curves of adsorption systems and to determine the characteristic design parameters of the column. The adsorption data were observed and modeled using the Adams-Bohart, Thomas, and Clark models. The models were used to predict the breakthrough curves of adsorption systems and to determine the characteristic design parameters of the column. The adsorption data were observed and modeled using the Adams-Bohart, Thomas, and Clark models. The models were used to predict the breakthrough curves of adsorption systems and to determine the characteristic design parameters of the column. The adsorption data were observed and modeled using the Adams-Bohart, Thomas, and Clark models. The models were used to predict the breakthrough curves of adsorption systems and to determine the characteristic design parameters of the column. The adsorption data were observed and modeled using the Adams-Bohart, Thomas, and Clark models. The models were used to predict the breakthrough curves of adsorption systems and to determine the characteristic design parameters of the column. The adsorption data were observed and modeled using the Adams-Bohart, Thomas, and Clark models. The models were used to predict the breakthrough curves of adsorption systems and to determine the characteristic design parameters of the column. The adsorption data were observed and modeled using the Adams-Bohart, Thomas, and Clark models. The models were used to predict the breakthrough curves of adsorption systems and to determine the characteristic design parameters of the column. The adsorption data were observed and modeled using the Adams-Bohart, Thomas, and Clark models. The models were used to predict the breakthrough curves of adsorption systems and to determine the characteristic design parameters of the column. The adsorption data were observed and modeled using the Adams-Bohart, Thomas, and Clark models.


TU388 Color removal of domestic sewage in urban poor communities by artificial wetlands.
Q.P. Amarango Jr, IFMA / PACA; T.C. Barros, R.T. Paulo, C. Rocha Jr, L. Moura
OMA; N.M. Brito, IFMA / DAQ; T.R. Franco, UFMA
One of the biggest environmental problems in cities of this size, is the water pollution caused by the disposal of domestic sewage. These effluents have high content of organic matter and nutrient load that can affect the natural dynamics of water bodies. This impact can transform aquatic environments on land, decreasing the supply of water, food and changing the dynamics of the landscape. An alternative to conventional procedures are called artificial wetlands or phytoremediation which are used in wastewater treatment to remove organic matter and nutrients, or for absorption of essential metals or stroke. These alternative methods may have advantages such as low cost of deployment and maintenance, little need for mechanical operation. The objective of this research was to improve the efficiency of removal treatment in this system of color based on studies of wetlands to be employed in low-income communities. We opted for the banana employment because they have better cost/benefit. Built a prototype pilot scale artificial wetland, in a residence, using plastic sheet for waterproofing and PVC pipes. The liquids were transferred after treatment of the reservoir by gravity, ensuring low operating costs. The effluent used with high load of organic carbon, phosphorus and nitrogen, was from the wastewater of a residence. There was previously a hole where residents cast solid waste. The place area was 13x17 m. The depth ranged between 70 and 100 cm. Plastic sheeting were placed for waterproofing. Six banana seedlings were planted. It was observed that the residents maintained practice to dispose solid waste in wastewater treatment area. We was told that they do not throw the waste treatment system. After three months of operation, five seedlings died. It was observed that the changes that remained was one that was not in the left trees, do not fall fruities over the next area to this change and the effluent of the contribution was related to that individual as well as being located in the region more depth. The seedlings that died were further from the effluent inlet, were shallower, there was shade and fruit trees fall directly on these seedlings. The taps were washed six times and percentage of the resin in the outlet pipe was removed by unknown and unauthorized person. There was no evidence effluent treatment efficiently due to the reported facts.

TU389 Aquatic humic substances and heavy metals
M. GONZALEZ GUADARRAMA, M. ARMIENTA HERNANDEZ, Universidad Nacional Autonoma de Mexico / RECURSOS NATURALES INSTITUTO DE GEOFÍSICA
Organic matter has an important role in the chemical speciation of heavy metals. Aquatic humic substances (ASH) are products of degradation of biomolecules and its structure is formed by monosaccharides, amino-acids and lignins. The complex formation between ASH and heavy metals depends on physicochemical conditions such as pH, redox potential, temperature and others. Environmental implications of this study are the analysis of mobility of As, Cu and Mn, with high ecological importance. The aim of this study is to calculate complexation constants under different conditions of pH, to compare the complexation degree. In addition, water metal reactivity in rain with humic substances was evaluated.

TU390 Dredging marine sediments in Catania harbour: options for a differentiated use
F. Pilato, ISPRA-Institute for Environmental Protection and Research; M. Secci, ISPRA Italian Institute for Environmental Protection and Research; S. Macchia, ISPRA Institute for Environmental Protection and Research; D. Sartori; L. Morroni, ISPRA Institute for Environmental Protection and Research; S. Giuliani, ISPRA; T. Cillari, V. Vitelli, B. Stancanelli, ISPRA Italian Institute for Environmental Protection and Research; d. Pellegrini, ISPRAInstitute for Environmental Protection and Research
Since 2004, the Institute of Environmental protection and research ISPRA is involved in a series of technical and scientific activities related to the dredging of marine sediments within the new commercial port of Catania, Sicily (Italy). In 2010, ISPRA started to carry out a new characterization of dredging materials with the aim to find sustainable solutions to allocate the dredged sediments, in alternative to the undifferentiated discharge at sea. Solutions were identified in agreement with the guidelines given by the technical- scientific "Manual handling of marine sediments ." APAT - ICRAM 2007 and based on the environmental data obtained over the last decade. Characterization and subsequent environmental monitoring activities were carried out using an integrated approach. In addition to traditional chemical and physical analysis, were performed Mussel Watch campaigns, study of benthos community and different batteries of bioassays (Paracentrotus lividus, Acartia tonsa, Pheodactylum tricornutum, Vibrio fischeri). The results of characterization allowed to identify different quality classes of materials, according to their physical, chemical and ecotoxicological properties. Then, a selective dredging was suggested to differentiate the final destinations of sediments to be removed (about 1.5 million cubic meters ), with clear socio-economic and environmental benefits. Three different solutions have been adopted: 1 - Reuse of sand within the building process of new docks and port

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yards: 350,000 m$^3$ of material were employed, otherwise extracted from quarries or land; 2 - Nourishment of beaches of the Catania gulf and subjected to erosion: 350,000 m$^3$ in 3 - Discharge at sea in a specific site authorized by the Environmental Minister: 800,000 m$^3$ representing the remaining material. In this study, management criteria and detailed results of the physical, chemical and ecotoxicological characterization of dredging materials are reported.

TU391

**Selected of sorption pharmaceuticals and personal care products (PPCPs) in agricultural soils**

N. Torres-Fuentes, c. corada, E. Gonzalez-Mazo, University of Cadiz / Physical Chemistry; D. Alvarez-Munoz; P. Lara-Martin, University of Cadiz / Agricultural Chemistry; A. Fermin, University of Cadiz / Soil Science. A wide range of pharmaceuticals (17) and personal care products (PCPs) have been extensively used in recent decades. In this study, we have selected several PPCPs that have been previously detected by our research group in soils and water/soil ratios to compare the sorption capacity of these chemicals in agricultural soils from that sampling area. First, physico-chemical properties of soil samples were determined in the laboratory: pH, organic carbon content, water holding capacity and texture. Sorption assays were performed using the batch equilibrium method according to OECD guidelines (TG 106). Briefly, 80 mL polypropylene tubes were filled with soil (0.5 – 2 g) and HPLC water (50 mL) spiked with target compounds at concentrations from 0.1 to 500 ng/L. After agitation (soil/water ratio was reached within 48 h), both phases were separated by centrifugation and determination of the concentration of the analytes was carried out by liquid chromatography - time-of-flight - mass spectrometry (LC-ToF-MS). Data for each pharmaceutical were fitted to a Freundlich isotherm model ($R^2$ > 0.95). Freundlich sorption constants (Kf) for the selected compounds ranged between 10 and 158 L/kg for HCTZ, 31 and 104 L/kg for MTP and 783 and 6045 L/kg for CLTM, 21 and 55 L/kg for MEF, 1014 and 5106 L/kg for TPT. Most of these PPCPs showed relatively high leaching potential, especially when compared with most well-known hydrophobic persistent organic pollutants (i.e., DDT). However, their sorption capacity may change dramatically depending on environmental conditions such as temperature (which was evaluated by calculation of temperature correction factors FT) or pH values as AFp. This was evident for those compounds such as CLTM, showing positively charged groups in their molecular structures (if pH > 6) that enhance their interaction with clay soils.

TU392

**Improved understanding of triphenyltin sorption on cultivated soils**

X. xiaoyu, School of Life Sciences

Abstract: Organotin compounds, especially triphenyltin (TPT), have been widely used as wood preservatives, pesticides and fungicides since the 1960s because of their high effectiveness toxicity, which resulted in a large number of TPT directly introduced into the soil and aquatic environment. Inadequate knowledge of TPT sorption has hindered an accurate evaluation of its environmental fate that is mostly complicated by the dual metal-hydrophobic nature of TPT. An improved understanding of TPT sorption and its influential factors is in need to fully assess the ecological risks of TPT in the soil environment. In the present study, TPT sorption on five Chinese cultivated soils, named black soil (A), calcareous soil (B), lateritic red soil (C), brown soil (D) and red soil (E), respectively, was estimated using the batch equilibration method as influenced by pH and biochar (BC) amendment. The results showed that the sorption isothers of TPT fitted well by linear equation in the five soil samples, implying partitioning is the dominant mechanism. TPT sorption increased with the increase of the organic carbon content on soils with their sorption coefficients ($K_s$) in the sequence of A (1708.6-4194.7 L/kg) > B (276.2-862.1 L/kg) > C (288.3-155.3 L/kg) > D (274.7-54.4 L/kg). In addition, the higher CEC of the soils may also enhance the TPT sorption. The changes of pH influenced the intensity of TPT, resulting in the maximum sorption of TPT occurred at pH 5.27 (the pK$_a$ of TPT) due to enhanced ion exchange. Moreover, TPT was one orders of magnitude more effectively sorbed by BC than by total organic carbon of A. BC fraction was considered to be the primary contributor to TPT sorption on soils. Key words: soil; triphenyltin; sorption; pH; biochar.

TU393

**Behavior of seven selected pharmaceuticals in thirteen different soils**

R. Kolesova, M. Kocarek, A. Klement, Czech University of Life Sciences Prague / Dept of Soil Science and Soil Protection; R. Grubic, University of South Bohemia in CB / Faculty of Fisheries and Protection of Waters, South Bohemian Research Centre for Aquaculture and Biodiversity of Hydrocences; M. Fer, Czech University of Life Sciences Prague / Dept of Soil Science and Soil Protection; O. Golovko, University of South Bohemia in CB / Lab of Environmental Chemistry

Biochemistry; A. Nikodem, O. Jaksik, Czech University of Life Sciences Prague / Dept of Soil Science and Soil Protection; V. Kodes, Czech Hydrometeorological Institute / Section of water quality Knowledge of contaminant behavior (e.g. its adsorption onto soil particle, degradation etc.) is essential when assessing contaminant migration in water environment. This study was focused on evaluating adsorption isotherms and half-lifes for 7 pharmaceuticals (clarithromycin, trimethoprim, metoprolol, atenolol, carbamazepine, sulfamethoxazole) on 13 soils of different soil properties. Adsorption of ionizable compounds was highly affected by soil pH. The adsorption coefficient of sulfamethoxazole was negatively correlated to soil pH and thus positively related to hydrolytic acidity and exchangeable acidity. Adsorption coefficients for clindamycin and clarithromycin were positively related to organic carbon and thus negatively related to hydrolytic acidity and exchangeable acidity and positively related to base cation saturation. Adsorption coefficients for the remaining pharmaceuticals (trimethoprim,metoprolol, atenolol, and carbamazepine) were also positively correlated with the base saturation and cation exchange capacity. Degradation rates in some degree reflected adsorption of studied pharmaceuticals on soil particles and increased with decreasing adsorption. The highest mobility in studied soil was observed for carbamazepine and positively related to sulfamethoxazole, but this pharmaceutical was relatively quickly degraded. The second highest mobility was found for carbamazepine, which mostly did not noticeably degrade during our experiments. Thus this pharmaceutical has the highest potential to migrate in water environment. The lowest mobility was observed for clarithromycin. However, this pharmaceutical due to its stability may be retained in the environment for long time.

TU394

**Environmental fate of a micro granular form of the insecticide cypermethrin in sunflower cultivated field plots**

N. Mantzios, Technological Educational Institute of Epirus / Faculty of Agricultural Technology; A. karakitsos, D. Hela, University of Patras; E. Leneti, Technological Educational Institute of Epirus; I. Konstantinou, Department of Environmental and Natural resources Management

A field dissipation and transport study of a micro granular form of the insecticide cypermethrin (CYP) has been conducted in a sunflower cultivation under Mediterranean conditions. The experimental field area (700 m$^2$) was divided in two groups of six plots each with a plot dimension of 4x10 m. One group of plots was used for sunflower cultivation when the other one as control plots (bare soil plots). Two different slopes (1% and 5%) were formed in each group. In the lower side of every plot a surface runoff collection reservoir was established. Soil samples were randomly taken at two depths: 0-10 and 10-20 cm. Runoff water and sediment samples were collected after rainfall or irrigation events. This was evident for those compounds such as CLTM, showing positively charged groups in their molecular structures (if pH > 6) that enhance their interaction with clay soils.

TU395

**Effects of forest fires on the levels and distribution of polycyclic aromatic hydrocarbons in soils and ashes**

I. Campos, Universidade de Aveiro / Department of Environment; N. Abrantes, Universidade de Aveiro / Institute of Environment Sciences and Technology; J. Kezer, Environment Research Centre for Environmental and Marine Studies CESAM University of Aveiro Aveiro Portugal; C. Vale, IPMA / Instituto Portugues do Mar e da Atmosfera; C. Micaelo, IPIMAR; P. Pereira, Department of Biology and CESAM Polycyclic aromatic hydrocarbons (PAHs) are widespread ubiquitous environmental contaminants, which had received major concern regarding public health and environmental impact due to their toxic, carcinogenic, mutagenic and teratogenic activity, as well as their environmental persistence and tendency for bioaccumulation. Although forest fires have been identified as an important source of PAHs little data is available concerning the effects of forest fires (including the role of ash) on the soil PAH content. To address this issue, we investigated the
temporal trends and profiles of fifteen priority PAHs according to the United States Environmental Protection Agency in soil and ash samples collected immediately, 4, 8 and 15 months after a forest fire (IAF, 4.8, 15MAF, respectively) in Ermita, North-centre of Portugal. Analyses of PAHs were performed on a gas chromatography-mass spectrometry (GC-MS). IAF the concentrations of PAHs in burnt soils were higher than in the corresponding non-burnt soil samples (70%). However, PAHs levels decreased considerably to those of the non-burnt soil. In the non-burnt soil, no temporal differences were recorded. The burnt soils and ashes were characterized by higher levels of light PAHs with three to four rings, reflecting direct emissions from biomass burning. Moreover, the three rings showed a pronounced decrease over time since-fire. For the non-burnt soils, phenanthrene (PHE) and fluoranthene (FLT) were the most abundant compounds, 20% and 16%, followed by pyrene (PYR) with 16%. For the burnt soils, we observed 40% of PHE, followed by FLT (15%) and PYR (10%). The ashes samples showed high fractions of PHE (35%), fluorene (FLU; 24%), FLT (10%) and PYR (10%), with a temporal decreased of FLU (14%) and a slightly increased of PHE (40%). The levels of PAHs in ashes were consistently enriched in PAHs relatively to corresponding soils, suggesting that soil were contaminated by ash-derived PAHs. Our results clearly suggest that PAHs produced by the forest fires seems to be adsorbed by soil and ash, being ash one of the main agent influencing the concentration of PAHs in the soil. Moreover, the decreased in PAHs levels over time, mainly due to the washout of the ash, may indicate that PAHs can be transport downstream by soil/ash runoff. This study point out the importance of further studies to assess the impact of PAHs in aquatic systems downstream to burnt areas. Keywords: Wildfire; PAHs; Assessment; Soil.

TU396 Characterization of a fuel contaminated site for the implementation of combined decontamination techniques R. Millan, CIEMAT / Department of Environment; M. RODRIGUEZ-RASTRERO, M. Sierra, T. SCHMID, F. DÍAZ-PUENTE, N. AREVALO, O. ESCOLANO, CIEMAT / Environmental
Fuel spills is a global concern problem that affects soil and water and need of an innovative an environmental friendly solutions. When a heterogeneous media is affected, the combination of different technologies could be recommended. In this framework, the Life Bioxsoil Project (Life ENV 11/ES/505) develops a combination of chemical and biological based technologies to be applied in an aged contaminated site located in SW of Spain. The complex characteristics of the site, an operative industrial facility in a saltmarsh area within a military area had required of an exhaustive characterization of 1.5 Ha to be treated. Physicochemical and biological parameters have been studied in surface and in depth, taking into account the bioavailability and those of the leachates. The obtained results show a wide range of THPs affection (in surface and in depth); salt content variability and high calcium carbonate amount among others. Furthermore, this area shows a relevant seasonal influence and a critical anthropogenic impact. The selected techniques are: “in situ” chemical oxidation, flitotechnology and enhanced natural attenuation that have been adapted taking into account these specific characteristics and are currently been tested simultaneously. The obtained data will be the base in order to develop an adequate protocol to recuperate this site and can be extrapolated to similar contaminated areas. Keywords: Fuel contamination, site characterization, remediation

MINETOX is coordinated project financed by the Ministerio de Economía and Competitividad of Spain that aims to evaluate the effect of biochar from urban wastes and sewage sludge on the fate and toxicity of mine wastes. The project comprises three sub-projects developed by Spanish research centres: Universidad Politécnica de Cartagena (subproject CGL2013-49009-C3-3-R), Universidad de Almería (subproject CGL2013-49009-C3-3-R), and Acondicionamiento Terrassenase-LEITAT (subproject CGL2013-49009-C3-2-R), with the collaboration of the VU University, Amsterdam. The overarching aim of this project is to assess the toxicity of mine tailings from old mine exploitations before and after amendments with different types of biochars. To achieve this objective, this study will address the following aspects: * The viability of the soil seed bank from the mine tailings area will be studied, and further recommendations pointing at a recovery of the vegetation cover will be established. * Column experiments with acid and basic mine wastes will be set up. The treatments tested include additions of different (types of) biochars, presence/absence of plants, and changing hydric conditions in order to assess the metals leaching. The pH and redox potential (Eh) will be regularly monitored, and soil pore water and lixiviate samples extracted from the columns to determine their metal(loid) concentrations before and after the amendments. * The biogeochemical mechanisms implied in the role of biochar to immobilise metal(loid)s will be studied by means of specific equilibrium trials with mixtures mine wastes vs. biochar. * The effectiveness of the treatments to reduce the toxicity of the soil pore water, lixiviates and the solid phase will be evaluated by means of a battery of ecotoxicity tests which include seeds germination, plantlets development, genotoxicity, and earthworms and enchytraeids survival and reproduction. * At the end of the experiment, the plants will be collected from the columns and concentrations of metal(loid)s in leaves and roots analysed to evaluate the effect of biochar on its absorption and translocation. In addition, the stress of the plants will be assessed by physiological markers. Finally, a sequential extraction of soil metal(loid)s will be performed in order to determine how the different treatments influenced its speciation and to relate it with its fate, bioavailability and toxicity. The poster will present the outline and first results of the project.

TU398 Reduction in phytotoxicity of hydrocarbon contaminated soil by remediation using agricultural wastes D.D. Davis, Newcastle University / Civil Engineering and Geosciences; M.D. Jones, Newcastle University / School of Civil Engineering Geoscience; L. Singleton, Newcastle University / School of Biology
The growing interest in compost materials demand the measuring of their toxicity to determine if human risk-based criteria are met. As a result, plant sensitivity is increasingly being used as a bioindicator in the assessment of the efficacy of remediation treatments. This study uses phytotoxicity tests to assess the ecological health of coal tar-contaminated soil which has undergone bioremediation by composting with agricultural wastes (spent mushroom compost and straw) as feedstock. The total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) concentrations where measured before, during and after the composting duration. Corn, pea and mustard seeds were used for testing seed germination and plant growth of the pre- and post-composted matrix. After 56 days of composting, the TPH concentrations were significantly reduced by 78% to 38% of the original concentration, while those of the PAHs were reduced from 100% to 60%. Combining fertility of the compost matrices, the germination rate increased by 78% and 33% for the corn and pea seeds, respectively, 5 days after planting and 92% for the mustard seed 3 days after planting. The results generated from the chemical and toxicity assays revealed that the health of the contaminated soil was significantly improved after the compost-bioremediation.

TU399 Oxyfluorfen dissipation and its impact on microbial communities in sunflower field study cultivation N. Mantzos, Technological Educational Institute of Epirus / Faculty of Agricultural Technology; S. Nikolaki, University of Patras / Department of Environmental and Natural resources Management, University of Patras; G. Tsaias, University of Patras / Environmental and Natural resources Management; K. Bourtizis, University of Patras / Department of Environmental and Natural resources Management
A field dissipation study of oxyfluorfen (OXY) herbicide and its impact on soil microbial communities has been conducted in a sunflower cultivation under Mediterranean conditions. The experimental field area (silty clay soil) was divided in two groups of six plots each of 4x10 m² area. One group of plots was used for sunflower cultivation while the other one was the group of control plots. Two slopes (1% and 5%) were carved out in each group. Soil samples were randomly taken at two depths (0-10 and 10-20 cm). OXY was applied pre-emergence as a water emulsion of a commercial EC formulation at a rate of 233 g/ha. The dissipation of OXY in soil was monitored for a period of 191 days. The initial OXY concentrations (3 h after application) ranged from 0.142 (cultivated 1 slope) to 0.149 µg/g (uncultivated 5% slope). The half-life in the cultivated plots using first-order kinetics was determined to 45 and 46.5 days for slopes 5% and 1%. In uncultivated plots the respective values were 50.9 and 52.9 days. The impact on soil microorganisms has been assessed at population level by deploying a 16s rRNA Illumina amplicon sequencing approach. Pyrotag assays were carried out using universal primers U341F/805R targeting the V3-V4 variable regions. 16s rRNA sequences were analyzed as follows: (a) the reads were trimmed to remove sequencing adaptors, low quality base calls (≤ 30 Phred score) and size-selected (between 150 and 500 bp) using the QIME pipeline filtering scripts, (b) high quality sequence reads that have not be flagged as chimeras have been clustered into operational taxonomic units (OTUs), based on a sequence identity threshold of 97%, using Uclust, (c) drawing of one sequence for each OUT as representative was performed and then aligned to the Greengenes database using PyNast, (d) the
sequence representatives of each OTU has been taxonomically classified by BLASTn-based comparisons to the Greengenes and Silva databases within QIIME. The resulting set of OTUs will be used in diversity analyses. This approach will enable us to characterize in detail the impact of the OXY on the soil bacterial community.

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TU400
MAGNETIC EFFECT OF HUMATE ON MICROMYCETE COMMUNITIES IN COPPER CONTAMINATED SOIL
V. Terekhova, Institute of Ecology and Evolution RAS / Lab Ecological Soil Functions; A. Ivanova, Lomonosov Moscow State University; E. Fedoseeva, A.N. Sverstov Institute of Ecology and Evolution; M. Akulova, Moscow State University / Department of Soil Science; K. Hydralieva, Institute of Chemistry and Chemical Technology; O. Yakimenko, Mosco State University / Soil Science Faculty

In contaminated soils within a micromycetes complex the depletion of species composition, the increase of eurytops’ part and the species which are not authentic to the zonal soil conditions, and also the raise of potentially pathogenic species occur. Humic substances (HS) are used as agents for remediation of contaminated soils. They are abundant in non-arable contaminated agricultural areas and not organic inoculants in the environment may have positive effect on species composition of micromycetes communities. It was shown in the decline of the part of potentially pathogenic fungi included in the list of Biosafety Levels BS1 and BS2 (http://www.abs.org) in BS1. In the case when lower metal concentration (264 mg/kg) and humate (0.1 and 1.0 g/kg) presented in growth medium the part of pathogenic fungi included in both levels decreased. However, in the samples with combined addition of higher metal concentrations (528 mg/kg) and humate in both concentrations was noted a slight increase in the proportion of fungi included in the list of BS2. Thus, it was shown that copper leads to qualitatively and quantitative changes in cultivated fungi and organic inoculants of humate able to mitigate these consequences to some degree, but not completely. Acknowledgements: This study is supported by Russian Foundation for Basic Research (12-04-01230a) and ISTC (Project # KR-2092).

TU401
Is Eisenia fetida a suitable species to monitor environmental soil genotoxicants?
S. Lemière, Fonctionnement des Écosystèmes Anthropisés; A. Deram, University of Lille 2 / Laboratoire des Sciences Végétales et Foncières faculté de Pharmacie; F. BERNARD, LGCE, Universite de Lille 1 / Laboratoire des Sciences Végétales et Foncières; A. Lepetre, LGCE - EA 4551; F. VANDENBULKE, LGCE, Universite de Lille I / LGCE EA Univ Lille Fonctionnement des Écosystèmes Terrestres Anthropisés Cité Scientifique SN Rdc F Villeneuve d’Ascq France

As well as for air and waters, not only chemical analyses have to be undertaken to account to monitor anthropic contamination of soils. Biological responses of exposed organisms should be considered too and particularly for environmental genotoxicants such as heavy metals. The long-term effect of Cu contamination on the organism was determined by the presence of Cu and humate in the growth medium caused a significant change in the community composition of cultivated fungi. It was primarily indicated by low values of the Sörensen-Chekanovsky index in comparison with the control samples. The addition of Cu and humate in different degrees stimulated the growth of metal-resistant genera Aspergillus and Penicillium, dark-colored genera Chaetomium and Paraconiothyrium and potentially pathogenic Fusarium. At the same time the addition of any components resulted in the elimination of genus Pseudogymnoascus. Humate had a positive effect on species composition of micromycetes communities. It was shown in the decline of the part of potentially pathogenic fungi included in the list of Biosafety Levels BS1 and BS2 (http://www.abs.org) in BS1. In the case when lower metal concentration (264 mg/kg) and humate (0.1 and 1.0 g/kg) presented in growth medium the part of pathogenic fungi included in both levels decreased. However, in the samples with combined addition of higher metal concentrations (528 mg/kg) and humate in both concentrations was noted a slight increase in the proportion of fungi included in the list of BS2. Thus, it was shown that copper leads to qualitatively and quantitative changes in cultivated fungi and organic inoculants of humate able to mitigate these consequences to some degree, but not completely. Acknowledgements: This study is supported by Russian Foundation for Basic Research (12-04-01230a) and ISTC (Project # KR-2092).

TU402
Aided phytostabilisation: Influence of fly ashes on metal mobility, microbial viability and oxidative stress of trees grown in Pb, Cd and Zn highly contaminated site
A. Verdin, Université du Littoral Côte d’Opale / Unité de Chimie Environnementale et Interactions sur le Vivant UCEIV EA; J. Fontaine, S. Firmin, S. Labidi, Université du Littoral Côte d’Opale; F. Douay, LGCgE, Université du Littoral Côte d’Opale

Combined use of in situ stabilization and energy production from forest biomass is a cost effective technique being explored to confront the problem of phytom amendment of metal contaminated sites. Between different amendments studied, Coil Fly Ash (CFA) has been shown to reduce metals mobility and to limit physico-chemical characteristics modifications of the soils due to the afferentation. Moreover, others studies revealed CFA efficiency to impair metal accumulation and to alleviate stress in several plants species. However, little is known about the plant health promoting action of these amendments, especially benefits on tree species growing in TE polluted sites. This in situ study aimed at evaluating the long-term effects of CFA as metal immobilizing agent in aided phytostabilization of trees cultivated in Pb, Cd and Zn contaminated soils. The effect of the metal (the activity of the Metaluerop Nord smelter (Noyelles-Godault, North of France), using four tree species (Alnus glutinosa L., Acer pseudoplatanus L., Salix Alba L., and Robinia pseudacacia L.). Before being planted with a tree mix in 2000, the site was divided in a reference plot (R) with no amendment and two plots amended with silica-alumimous (FA1) and sulfo-calcare fly ash (FA2). Eleven years after plantation, vegetation surveys (phytotoxicity status, phytostabilization activity) were performed using an urban contaminated soil sample. The resulting set of OTUs will be used in diversity analyses. This approach will enable us to characterize in detail the impact of the OXY on the soil bacterial community. The resulting set of OTUs will be used in diversity analyses. This approach will enable us to characterize in detail the impact of the OXY on the soil bacterial community. The resulting set of OTUs will be used in diversity analyses. This approach will enable us to characterize in detail the impact of the OXY on the soil bacterial community.

Acknowledgments: This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARCHIMEDES II. Investing in knowledge society through the European Social Fund.

TU404
Polyurethane Rigid Foam from Vegetable Polyol with Residual Inorganic Load Applied to Removal of Oleophilic Contaminants
V.R. Silvy, O.S. Santos, Federal University of Minas Gerais / Chemistry Department; M.C. Silva, Federal University of Itajubá / Materials Engineering; M.I. Yoshida, Federal University of Minas Gerais / Chemistry Department

Solid extractor materials have emerged as a powerful tool in extracting pollutants in water, among these materials Polyurethane Rigid Foam (PUF), which has gained importance in the recent times, as solid adsorbents in toxic substances sorption processes. Due to the high porosity and presence of potential active sites such as amine, hydroxyl and hydroxyal, and hydophobic hydrocarbon chains, PUF is present as a versatile alternative to removal of hydrophilic as well as hydrophobic substances. Regarding to fats and oil removal, are considered good absorbent materials that show hydrophobic groups and high rates of absorption and retention of oleophilic substances. The PUF presents these important characteristics. This paper presents some results of the use of polyurethane rigid foams (PURF), with and without inorganic filler, as car engine oil absorber. The PURF used in this study were synthesized from vegetable oil (castor oil, widely used in tropical countries as a food oil, plant based polyols, and other monomers). The second type of PUF (PUF + P3 Residue) as a filler is an attempt to contribute to reduce the environmental impact caused by the accumulation of industrial waste and add value to a residual material. The absorption studies showed a three fold increase in the load foam sorption capacity, when compared with foams without inorganic filler. The introduction of waste as inorganic filler, besides improving the flame retardancy characteristics of the foams, increased their average cell size of about 40%. Despite no oleophilic characteristics of the inorganic filler, the increase of average cell size and open/closed cell contributes to higher oil absorption by the foam.

TU404
REMEDIATION POTENTIALS OF PLEUROTUS FLORIDA ON SPENT LUBRICATING OIL CONTAMINATED SOIL

P. flordia, University of Badan, Nigeria / crop protection and environmental biology

Soil and surface water contaminations by spent lubricating oil (SLO) are common occurrences in most developing countries where agriculture is the mainstay of rural inhabitants, especially in the oil producing ones. This is rapidly increasing due to the global rise in use of petroleum products. The white-rooted Pelargonium, Pleurotus Florida was investigated for its ability to mineralize heavy metals, improve soil nutrients and reduce the Total Petroleum Hydrocarbon (TPH) in spent lubricating oil contaminated soil after 2 months. 4kg of top soil was thoroughly mixed with 100ml, 200ml, 400ml, 800ml and 1600ml of the oil to give 2.5%, 5%, 10%, 20% and 40% contamination levels respectively and a set of control was kept (0%). 10g of vigorously growing spawn of the fungus was added and observed at 1 and 2 months of incubation. After 2 months, the total petroleum hydrocarbon (TPH) reduced from 265.33mg/kg to 232ml/kg for 10% contaminated soil and from 52.33ml/kg to 35ml/kg for 2.5%. Nutrient contents were high at 1 month, followed by a decrease after 2 months. Levels of heavy metals like Mn, Ni, Pb, Cr and Zn fluctuated at 1 month but were followed by significant decreases after 2 months. The levels of Mn and Ni reduced from 9.53mg/kg to 8.18mg/kg, and 9.51mg/kg to 8.23mg/kg respectively for 40% contamination by the end of 2 months. The improvement of nutrient content value, bioaccumulation of heavy metals and reduction of TPH at all levels of SLO concentrations tested through inoculation with P. flordia is of importance for mycoremediation of spent oil polluted soils. Key words: Total Petroleum Hydrocarbons, remediation soil.

TU405 PHYTOREMEDIATION OF METALS AND PETROLEUM PRODUCTS FROM INDUSTRIAL WASTEWATER OF PLANTS AT RLC, QATAR

M.Y. Al-Sulaiti, ExxonMobil Qatar Research & Emission Control; E.M. Abdel Bari, Environmental studies Center-QU; C.S. Warren, ExxonMobil Qatar Research & Advanced Environmental Scientist; T.M. Al-Shaikh, Environmental Studies Center-QU

Qatar is an arid country with limited water resources. LNG activities generate considerable amount of industrial wastewater (IWW). Turning this nuisance into beneficial resource will contribute to sustainable environmental economy and development and facilitate disposal alternatives. Phytoremediation is in use in many countries. It can clean soils and water. The objectives of the native plants experiments were: 1- Set up a system of native plants growth in soil, sand or /and water using IWW, 2- Study the impact of the IWW and the accumulation of organic and inorganic components on growth of some native species, and 3- Identify some microorganisms in IWW or soils that can play a role in the bioremediation processes. Plants growth in sand media containing IWW greatly inhibited seed germination and the subsequent growth of seedlings of Medicago sativa, Hordeum vulgare, and Chloris gayana. Growth of Phragmites Australis rhizoshomes and Sporobolus icilados were hindered considerably. Plants growth in soil, on the other hand, was different from that in sand. The reduction in the germination of barley seeds was considerable and the prophyl of most plants became dry as a sign of toxicity. Growth of seedlings of alfalfa and Rhodes and rhizomes of Sporobolus icilados was slightly affected in soil plots irrigated with IWW. On the other hand reeds seemed promoted by IWW. Significant reduction in the photosynthetic pigments and soluble sugars was caused by IWW in plants studied. Proline, as a stress indicator, did not show any consistency with IWW treatment. Such findings were confirmed by using IWW in combination of Apirolio and municipal solid waste compost. Microbiological and chemical analyses were carried out at different times in order to evaluate the changes in the structure and function of microbial populations in relation to the different experimental conditions.

TU407 Phytoremediation strategies for dehydroxylation and quality improvement of a maraschino digestive sludge

P. Genni, National Research Council of Italy (CNR) / Water Research Institute; S. Taricco, National Research Council of Italy / Water Research Institute; M. Di Lenola, National Research Council of Italy / Water Research Institute; S. M. Al-Nasir, National Research Council of Italy / Water Research Institute; M. Di Lenola, A. Barra Caracciolo, National Research Council / Water Research Institute; A. Massacci, National Research Council of Italy / Institute of Agro Environment and Forest Biology; P. Sconocchia, ARPA Umbria / Serzione per la Protezione Ambientale; D. Liberati, Liberti; S. Cologni, Entitá degli studi della Tuscia - Viterbo; P. De Angelis, Universitá degli studi della Tuscia - Viterbo / Dipartimento per la Innovazione nei Biologi Agroalimentari e Forestali DBFAB

An artificial pond (about 2.6 hectares and 4 meters depth) was filled with the liquid and solid fine residual fraction from a biogas system located in an agricultural area (Central Italy). The resulting sludge consisted of about 80% of water; the fine fraction (20%) was characterized as: sand 15%, silt 60% and clay 25%; without any significant vertical stratification in the pond. The pH and the electrical conductivity were about 8 and 2.5 mS cm⁻¹, respectively. The heavy metals detected at high concentrations were copper (1200 mg kg⁻¹) and zinc (4000 mg kg⁻¹). Zoodoom Project, funded by Umbria Region (Italy), aimed to test a green remediation technique for the treatment of sludge contaminated with heavy metals, by using the aquatic phytoremediation. A phytoremediation pilot system was set up in a greenhouse for testing the effectiveness of different plant species and planting methods for sludge dehydroxylation, as well as the quality improvement of the sludge. The tested plants (cuttings or rooted cuttings) were Tamaris gallica, Tamaris africana, Phragmites australis and a combination of Juncus effusus, Iris pseudacorus and Carex gracillis. The plant growth, the sediment water content, the main physico-chemical parameters and the pathogen occurrence in the sludge (E. coli and Enterobacteriaceae) have been analyzed in the greenhouse experiment. At the same time, the evolution of the microbial community after the plant addition has been evaluated by measuring its abundance, viability, activity (dehydrogenase activity) and diversity (Fluorescence In Situ Hybridization). The results of the first year experiments are here reported.

TU408 Phytoremediation process for contaminated soils: a life cycle assessment approach

C. Sablayrolles, INP-ENSIACET, L. Tanfin, INP-Toulouse (Ensiaet) / Laboratoire de Chimie Agro industrielle; C. Vialle, Université de Toulouse / INPENSIACET LCA Laboratoire de Chimie Agro industrielle INRA UMR CAI; G. Vilarem, INPToulouse Ensiaet / Laboratoire de Chimie Agro industrielle; J. Kallhoff, Ecolab / Ecolab Laboratoire déodologie fonctionnelle et environment; L. Thannerberg, VALGO

Comparative life cycle assessment of a number of brownfields from former industry remain in France, presenting health and environmental hazards. In the current context, where conventional treatment methods (ex situ or in situ) are expensive and energy intensive intensive energy intensive intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy intensive energy 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TU409 Maximisation of oil recovery from an oil sludge washing process with surfactants considering surfactant type and concentration, and surfactant to oil sludge ratio D.F. Ramirez Ocampo, University of Reading / Department of Geography and Environmental Science; C.D. Collins, Reading University / Soil Research Centre Oil sludges are a complex mixture composed mainly of crude oil, water and sediments. These hazardous wastes generated in the petroleum extraction and refining processes. Treatment of oil sludges to date has been focused on physicochemical remediation and bioremediation techniques. Oil recovery methods have been applied for obtaining the extracted oil for reuse, and oil sludge washing (OSW) with solvents are being used in this study. This method consists of using surfactants in order to remove and recover the oil from the sludge matrix. This study aims to maximise the oil recovery considering three important parameters in OSW: surfactant type and concentration, and the surfactant to oil sludge (S/O) ratio. Four synthetic surfactants [Sodium dodecyl sulphate, SDS (anionic); Tween 80 (T80), Triton X-100 (TX100), and Triton X-114 (TX114) (non-ionic) and one biodegradable surfactant (raffinose) used in this study. These surfactants were characterised before OSW. Characterisation included micelle size determination by dynamic light scattering (DLS), critical micelle concentration (CMC) determination by the pendant drop method, and surface activity assessment by the oil displacement test. By confirming the CMC, absolute concentrations in terms of CMC were established for the oil sludge washing study. Toluenol was used as co-solvent in the OSW in order to aid in the separation of oil. Due to the high number of possible experimental runs in this study with three factors and five levels, a Taguchi design of experiment methodology was applied for the experimental design to reduce the amount of experimental runs from 125 to 25 per Taguchi replicate. Three replicates of the Taguchi orthogonal array were tested and the recycled oil was measured gravimetrically. S/O ratio was the factor with the largest effect. The levels with the maximum oil recovery were 1:1 of S/O5 ratio, 1CMC of surfactant concentration and TX100. Also, RL and TX114 showed a high effect similar to TX114. In conclusion, low surfactant ratios (1:1) and low surfactant concentrations (1CMC) can be used to reduce costs while maximising the amount of recovered oil. Further treatments may then continue with other remediation techniques to ensure that the sludge have contaminant concentrations below the legal limits if required.

TU410 Insolubilizing Effect of Heavy Metals in Soil by Calcium Polysulfide Y. Kewage, National Institute of Advanced Industrial Science and Technology (AIST), Tochigi, Japan Soil contamination by heavy metals such as arsenic or lead have been increasing in Japan. According to ministry of the environment government of Japan, the number of the groundwater or the soil contaminated site of heavy metals is 11.539 cases at the end of 2012. The main cause of those contaminates were natural origin heavy metals, which were widely observed around mine area or hot spring area. As the contamination area by the natural occurring heavy metals was widespread, an in situ treatment method such as insolubilization or bio-treatment would be effective in those area. In this study, leaching behavior of the heavy metals from soil by using calcium polysulfide were evaluated. A long-term soil leaching batch experiment were carried out under various environmental condition. The leaching behaviors were studied. It was measured gravimetrically. S/O5 ratio was the factor with the largest effect. The levels with the maximum oil recovery were 1:1 of S/O5 ratio, 1CMC of surfactant concentration and TX100. Also, RL and TX114 showed a high effect similar to TX114. In conclusion, low surfactant ratios (1:1) and low surfactant concentrations (1CMC) can be used to reduce costs while maximising the amount of recovered oil. Further treatments may then continue with other remediation techniques to ensure that the sludge have contaminant concentrations below the legal limits if required.

TU411 Bioremediation of benzene, toluene, ethylbenzene, and xylene (BTEX) using non-pathogenic bacterium H. Kim, S. Lee, Kyonggi University Recent years have seen many studies on how to biodegrade poly-aromatic compounds as the benzene, toluene, ethylbenzene, and xylene using various methods. In this study utilizes high efficient non-pathogenic bacteria such as photosynthetic bacteria and/or LAB (lactic acid bacteria) for remediation of BTEX contaminated soil. Their non-pathogenic characteristics allows them to be a reliable solution for similar groundwater and soil contaminations due to oil spills. Purple non-sulfur bacteria, Rhodobacter capsulatus, showed efficient degradation of 20mg/L of BTEX. 1g/L (w/v) of cell mass was inoculated to MSM (Mineral Salts Medium 6.0) with different level of benzoyl-CoA reductase gene according to substrates. However R. capsulatus was expressed different level of benzoyl-CoA reductase gene according to substrates. As the leaching values of BTEX in 7 days. 1g/L (w/v) of cell mass was inoculated to MSM with 0.01 % yeast extract under facultative aerobic condition. Analysis of degradation efficiency was performed using headspace method via GC-FID (Gas Chromatography-Flame Ionized Detector). Gene expression experiments, such as real-time PCR were also used to identify and confirm the target gene responsible for the degradation of BTEX. Target genes were benzene monoxygenase (BMO), benzene dioxygenase (BED), toluene dioxygenase (TOD) and benzoyl-CoA reductase subunit A and B. Monodioxygenase and reductase were commonly located in the degradation pathway of BTEX. Extracted mRNA was converted to cDNA as template for real-time PCR. The R. capsulatus was expressed different level of benzoyl-CoA reductase gene according to substrates. However R. capsulatus did not expressed oxygenase gene. Based on this study, when non-pathogen bacteria LAB applied for remediation of BTEX contaminated soil, biologically risk was low. Further studies have to be performed to optimize and enhance the removal efficiency and it gives a gateway for the bioremediation of aromatic compounds.

TU412 Remedy Effectiveness Design Construct: Strategy for determining remedy effectiveness at large-scale contaminated sediment sites R.R. Otten, Middle Tennessee State University / Biology; D.M. Walters, USGS / Sevier County; J. Gilbert, C.D. Collins Science Center; J. Venable, P. Chau, U.S. Environmental Protection Agency / Great Lakes National Program Office Remedy effectiveness of contaminated sediments is focused on understanding the impact a specific remedy (e.g., dredging). Though simple in principle, designing such studies can quickly becomes very complicated due to multiple factors including multiple contaminants of concern, the location of the site, and complex river dynamics. In order to better design remedy effectiveness studies a Remedy Effectiveness Design Construct (REDC) was developed. This approach was developed specifically for contaminated sediment sites, however, the general principle may be adapted as necessary. The REDC consists of three main components: physical, chemical, and biological. The physical component focusses on the site factors that control the fate and transport of the contaminants. The chemical component focuses on concentrations in the surface water, pore water, sediment and biology. The biological component focuses on both laboratory and field-based indicators, including standard sediment and pore water toxicity tests, feral organisms, and passive samplers. Each of these components have inherent attributes that must be well understood before any proper experimental design can be constructed, such as which contaminant of concern is the priority and a good geospatial understanding of the site. The REDC provides a framework for ecotoxicologists working on remedy effectiveness of contaminated sediments sites and highlights the key factors and attributes necessary to include in the study design.

TU413 Vermicomposting as an advance biological treatment of industrial waste from leather industry (tanneries) R.R. Rachide Nunes, Universidade de São Paulo / Instituto de Química de São Carlos IQUQSC, R.M. Bontempi, G. Galetti, G. Mendoza, USP - Universidade de São Paulo / IQSCC Instituto de Química de São Carlos; M.d. Rezende, IQSCC-USP The leather industry (tanneries) generates high amounts of toxic wastes, including acid and liquid effluents, rich in organic matter and mineral content - with emphasis on chromium due to its carcinogenic effect. Without the correct treatment, when these residues reach soil, sediments, water bodies, and the atmosphere they could cause contamination of fauna and flora, disturbing their ecosystem. This work presents a vermicomposting method, silvicultura, and silviculture of sludge from liquid waste treatment station (VRC-S), and a mixture of both (VRC-CS). Vermicomposting was performed in 25 L plastic barrels and the amount of each residue was calculated based on the C:N ratio mixture, between 20-30. Five hundred earthworms (Eisenia fetida) were added into each barrel. During 135 days the following parameters were evaluated: pH, total organic carbon (TOC), organic matter (OM), cation exchange capacity (CEC), C:N ratio, and chromium content (Cr⁶⁺ and Cr⁴⁺) at times: 0, 15, 30, 45, 60, 90, and 135 days. As a result, the expectation that vermicomposting is an alternative for the treatment of tannery industrial waste was confirmed. Results of pH, TOC and OM content showed a decrease in its values during the processes, indicating the efficiency of the process;
The opposite trend, an increase for the values of CEC and total nitrogen also showed good results. The results of Cr<sup>3+</sup> and Cr<sup>6+</sup> were 8.05 and 0.78 mg L<sup>-1</sup> respectively in all treatments. At 135 days, all values of Cr<sup>3+</sup> were below the limit of quantitation. Therefore, Cr<sup>6+</sup> content probably was biologically transformed into Cr<sup>3+</sup>, confirming the use of this technique as an advance biological treatment. In all, this study reinforces the general idea that vermicomposting could be introduced as an effective technology applied to the treatment of industrial waste and production of agricultural inputs. As a future perspective, the vermicomposts will be applied to green bell peppers, evaluating in practice their fertilizing potential.

TU414
Which is the remediation degree of vermicompost from filter cake in contaminated soils by a metal smelter in Korea?
E. Benetti, L.B. Pigatin, IQSC-USP; M.M. Kanashiro, University of São Paulo; M. Rezende, Universidade de São Paulo / Chemistry
Bioremediation is a process in which living organisms are used to remove or reduce (ameliorate) environmental pollutants, especially organic compounds, and it can be used in places where the dopant is present. Bioremediation presents effective results in the medium term with a relative low cost. The aim of the study was to evaluate the degree of remediation of vermicompost in contaminated soils with glyphosate (the most widely used herbicide in the world) and deltamethrin (used for controlling flies and cockroaches). Acute toxicity, biomass and reproduction tests were performed according to ASTM, ISO 11268-1 and ISO 11268-2. Every test was performed with five repetitions, each result being the arithmetic mean of these replicates, and performed with soil collected in rural area. To evaluate the vermicompost remediation potential, 3% of vermicompost (w/w) was mixed to the soil. For glyphosate (GLY), 3 different samples (REFERENCE, SOIL + GLY and SOIL + GLY + FCV (filter cake vermicompost) were prepared. Already for deltamethrin (DELTA), two treatments were prepared (wih and without deltamethrin solution - the suspension without the active ingredient (DISP) was prepared by the manufacturer, for academic purposes), with 4 different samples (REFERENCE, SOIL + DISP or DELTA, SOIL + DISP or DELTA + FCV and SOIL + DISP or DELTA + OPV (orange peel vermicompost). In each vae, the humidity was maintained at 60%, and 10 adult earthworms were placed. To monitor acute toxicity, earthworms were weighed initially and on days 7 and 14. To monitor biomass, the weighing continued until 28<sup>th</sup> day. After that, the earthworms were removed of the vases and young earthworms were counted after 56<sup>th</sup> day. The results obtained in the toxicity tests were analyzed by the ANOVA and Dunnett test, with assurance of 95%. It can be observed that the addition of 3% of vermicompost in experiments involving glyphosate improves the development of the earthworms since it decreases the mortality rate, it improves the biomass gain and the number of earthworms that hatched. In the case of deltamethrin, the results show that the application of 3% of vermicompost on the medium is not effective, which is corroborated by the results obtained for the control. For the reproduction of earthworms is completely nullified, even in the presence of hummus in soil, so, a study should be developed to achieve similar results for the remediation of contaminated soils with deltamethrin.

TU415
Changes in physical and chemical properties of soil by soil washing of metal-contaminated soil
S. Jeong, Kunsan National University / Dept of Environmental Engineering; J. Kim, K. Ahn, Kunsan National University; Y. An, Konkuk University / Department of Environmental Science
There was a historic copper smelter in Korea, Janghsmelter. The Janghsmelter was operated from 1936 to 1989, emitting smelting gases to the vicinity of the gas stack. It has been known that soils near the smelter stack were highly contaminated with heavy metals, in particular lead, cadmium, and copper. In 2014, soil washing of the metal contaminated soils from the beginning of this year, 2014. There was a famous copper smelter in Korea, Janghsmelter. The Janghsmelter was operated from 1936 to 1989, emitting smelting gases to the vicinity of the gas stack. It has been known that soils near the smelter stack were highly contaminated with heavy metals, in particular lead, cadmium, and copper. In 2014, soil washing of the metal contaminated soils from the beginning of this year, 2014.

TU416
Priority contaminated site: Nubarakshen obsolete pesticides burial site (Armenia)
A. Aleksandryan, Hazardous Substances & Waste Policy Division / Head of Division; A. Khachatryan, Waste Research Center; V. Frangulyan, Center of Disease Control and Prevention
The problem of obsolete pesticides became urgent forArmenia since late 1970s. In 1980s a special place was allotted to bury them in the Nubarakshen obsolete pesticides burial site. Approximately 500 tons of obsolete pesticides were finally disposed of which 250 tons were organochlorine pesticides, mostly DDT. After thirty years of storage it was discovered that the burial is located in the zone of active landslides, which can damage the containers and obsolete pesticides may be released from the site. Later a secondary pollution occurred on the site, as some of the cells were opened. In 2010 the Ministry of Nature Protection performed activity on remediation of the burial site. To assess the degree of remediation of site soils were taken. The burial site localization was taken into consideration and average samples were analyzed for organochlorine POPs pesticides using Gas-Chromatography/Mass-Spectrometer GCMS-QP2010 (Shimadzu Corporation,Japan). Soils from the outward nearest plots on the average contained 1.4 mg/kg DDT, while soils from the same plots, if taken from the depth of 20 cm, contained on the average 2.3mg/kg DDT. Soil taken directly from the burial site contained about 59 mg/kg DDT, while soil near the drainage tube outgoing from the burial averaged to 13.5 mg/kg. Below the burial, at the fence from the outward part, at the depth of 1 m soil contained on the average 1.6 mg/kg p,p’-DDE and 6-8 mg/kg p,p’-DDT. In the same distance at a distance of 20-100 m the amount of DDT made appropriately 1.1 and 0.2 mg/kg. At the plots of summer-houses below the burial (>500 m) the residual amount of p,p’-DDE made 0.008 mg/kg, whereas p,p’-DDT = 0.0 mg/kg. In samples of soils taken at the near-by settlements Mushavan and Jranshen the amount of DDT on the average, made from 0.005 to 0.01 mg/kg. The conclusion was drawn: pollution was caused by disintegration of the burial site and the follow-up recovery works. As a result, remediation of an ineficient environmental factor incluing human health. High concentrations of volatile aromatic hydrocarbons, especially benzene (IARC group 1 carcinogenic), in urban areas produced mainly by traffic, may impose serious human health risk. Ambient air monitoring program in the City of Novi Sad was conducted by Institute of Public Health of Vojvodina. Monitoring of BTEX (benzene, toluene, ethylbenzene, xylene) in 2013 included five sampling sites: A, B, C, D, and E. From the results, it can be stated that the ambient air is an important environmental factor influencing human health. High concentrations of volatile aromatic hydrocarbons, especially benzene (IARC group 1 carcinogenic), in urban areas produced mainly by traffic, may impose serious human health risk. Ambient air monitoring program in the City of Novi Sad was conducted by Institute of Public Health of Vojvodina. Monitoring of BTEX (benzene, toluene, ethylbenzene, xylene) in 2013 included five sampling sites: A, B, C, D, and E. From the results, it can be stated that the ambient air is an important environmental factor influencing human health.
contaminated with PAHs, oxy-PAHs and N-PACs were obtained from 6 locations in three countries: a) a former gasworks plant in Karlstad, Sweden (5 samples); b) a former wood tar production site outside Stockholm (Riksten), Sweden (10 samples); c) a coke oven plant in France (3 samples); d) a coke oven and metallurgy site in France (1 mixed sample); e) a gasworks site in France (1 mixed sample); f) a gasworks site in Belgium (1 mixed sample), g) and a wood preservation site, Hvidovre, Denmark (1 mixed sample). In addition, with these soils we determined the total soil concentration and performed an analysis of the partitioning behaviour, and conducted worm bioaccumulation bioassays (with E. crypticus according to ISO 16387), enabling an analysis of biotake and bioavailability. Porewater concentrations, in combination with total soil concentrations and worm concentrations (Enchytraeus crypticus) agreed with expectations from independent partitioning and bioaccumulation models. Furthermore, we disagreed with recommendations of risk assessments in Europe and the United States, these are based on reference soils, and the soils on this study, coming from contaminated areas, are quite unique in their sorption properties compared to reference soils. For instance, the organic carbon in contaminated areas sorbs similarly to coal tar, but in reference soils sorbs similarly to humic acids. The results call into question the conservative nature of existing soil risk guidelines.

TU419
Bioelectrochemical systems for sustainable bioremediation
J. Monier, O. Sibour, ENOVEO
The recent discovery of microorganisms that can directly use electrodes as a sink or as a source of electrons has led to the development of innovative approaches for the remediation of polluted environments. Bioelectrochemical systems are emerging technologies which use microorganisms to catalyze oxidation reactions at an anode and/or reduction reactions at a cathode where pollutants serve as electron donors or electron acceptors. Accelerating remediation processes using such technologies has been demonstrated for several pollutants including reduction of toxic metals and chlorinated compounds, oxidation of aromatic hydrocarbons, phenol and potentially for any contaminants that microbes have been shown to anaerobically oxidize. This presentation will describe, through different case studies, how such systems work, their advantages and how they can be implemented in order to optimize or design new remediation strategies. Electrode-based strategies used to monitor microbial activity in real time will also be addressed.

Adverse outcome pathway concept in research and risk assessment (P)

TU421
Towards animal free human safety assessment - Adverse Outcome Pathway descriptions as a backbone for integrated testing strategies
T. Gocht, University of Tuebingen / Institute for Experimental and Clinical Pharmacology and Toxicology; E. Berggren, European Commission Joint Research Centre (JRC); I. Institute for Health and Consumer Protection BIPC and EU Reference Laboratory for Alternatives to Animal Testing EURL ECMV, M. Schwarz, Austrian Agency for Health and Food Security / Department for Data Management; M. Whelan, European Commission DG Joint Research Centre / European Union Reference Laboratory for Alternatives to Animal Testing EURL ECMV
SEURAT-1 is a major European research consortium that was established to develop the science needed to evaluate the safety of chemicals for repeated exposure in humans without using animals. Inspired by the fundamental considerations published in the report of the U.S. National Research Council (NRC) entitled ‘Toxicity Testing in the 21st century: A Vision and a Strategy’, SEURAT-1 aims to use the mechanistic understanding of toxicological effects for the development of innovative testing methods and, ultimately, improved safety assessment. A research strategy was formulated based on the guiding principle to adopt a toxicological mode-of-action framework to describe how any substance may adversely affect human health. The proof of the initiative will be in demonstrating the applicability of the concepts on which SEURAT-1 is built. This is done on three levels: (i) theoretical prototypes for adverse outcome pathways were formulated based on knowledge already available in the scientific literature on investigating the toxicological mode-of-actions leading to adverse outcomes (addressing mainly liver toxicity); (ii) adverse outcome pathway descriptions are used as a guide for the formulation of case studies to develop integrated testing strategies for the prediction of certain toxicological effects, which also as a consequence might further elucidate the theoretical model; (iii) further case studies targeting the application of knowledge gained within SEURAT-1 in the context of safety assessment. The ultimate goal would be to perform safety assessment based on ab initio predictions grounded on a sufficient understanding of toxicological mechanisms. In the near-term, it is more realistic that data from innovative testing methods will support in strengthening read-across arguments.

Midpoint or single score for decision making? (P)

TU422
How to communicate environmental impacts? Approaching LCA results to consumers for urban food products
e. sanye-menguil, Institute of Environmental Science and Technology ICTA; J. Oliver-Solá, Universitat Autònoma de Barcelona / Inèdit Innovació; J. Montero, IRTA; J. Rieradevall, Institute of Environmental Science and Technology ICTA Universitat Autònoma de Barcelona / sostenipra
Labeling schemes provide consumers with data to include environment as a decision-making criterion in purchasing, such as energy consumption and carbon emissions. Notwithstanding the easier communication through single indicators, the use of them can cover up other environmental impacts. In Life Cycle Assessment (LCA), impact assessment methods vary from single endpoint indicators to methods with a set of almost 20 midpoint indicators. But, what is the equilibrium between consumers’ understanding and the reliability of LCA results? Urban food production in new Roofop Greenhouses (RTG) is used as case study to test the comprehension of different LCIA indicators among consumers. The study aims to compare consumers’ preferences with LCA results for multiple indicators (both midpoint and endpoint). First, surveys will be done to consumers of the university community that will participate and consume local lettuces from the RTG-Lab (Universitat Autònoma de Barcelona, in Bellaterra, Spain), during the consumption harvesting cycle to be able to identify the environmental indicators that consumers perceived as preferable. Second, lettuce production will be assessed from a cradle-to-farm gate perspective through different LCIA methods. Consumers’ preferences will be contrasted to LCIA results to observe whether significant environmental impacts of products are hidden through certain indicators.

Alternative approaches for ecotoxicity assessments (P)

WE001
Combined ecotoxicological and hydrological methods for on-line contamination event detection in water distribution systems
L. Nuesser, RWTH Aachen University, Institute for Environmental Research / Institute for Environmental Research; S. Steffens, RWTH Aachen University, Institute for Environmental Research / Institute of for Environmental Research; R. Dorner, RWTH Aachen University / Institute of Hydraulic Engineering and Water Resources Management; M. Brinkmann, RWTH Aachen University, Institute for Environmenta / Institute for Environmental Research; T. Seiler, RWTH Aachen University / Ecosystem Analysis; C. Cofalla, RWTH Aachen University / Institute of Hydraulic Engineering and Water Resources; E. Salomons, N. Oliker, Technion Israel Institute of Technology; H. Schuettrumpf, RWTH Aachen University / Institute of Hydraulic Engineering and Water Resources Management; A. Ostfeld, Technion Israel Institute of Technology; H. Hollert, RWTH Aachen University / Institute for Environmental Research
Drinking water quality and availability are key elements of water security and may be compromised by instances of accidental or deliberate contamination. Hence, it is required to detect, identify and manage any negative, unforeseen and sudden change in drinking water quality to ensure urban water security and management. The collaborative project W²-Hydro combines ecotoxicological and hydrological methods and aims to (i) detect, understand and finally numerically simulate the governing physical, chemical and biological processes underlying a peak contamination and (ii) to set-up a risk based management framework for event detection. Within the project two risk scenarios are investigated, in order to simulate contaminations in water basins, the ecotoxicological scenarios of contaminated sediments were conducted and for event detection in water distribution systems two on-line bio-units were developed. Native sediment spiked with permethrin, cadmium and quinoline, respectively, in environmental concentrations was monitored using a water sediment system in an annular flume. The hydrotoxicological experiments provided data about bed shear stress, temperature, dissolved oxygen, pH value, conductivity and redox potential and suspended particulate matter. This enabled the detection of interactions of relevant parameters in the system after the remobilization of contaminants from the sediment. In order to detect contamination events in water distribution systems two on-line bio-units with high sensitivity and accuracy were developed. The combination of two biological monitors allows for the verification of alarm signals from the first instrument with the signal of the second, thereby reducing false alarm rates. Both bio-units were developed to use Danio rerio (zebrafish) embryos and larvae until the age of 120 hours post fertilization (hpf) as an alternative to animal testing. The first bio-unit is a custom designed flow-through well plate in which 72 hpf to 120 hpf zebrafish embryos and larvae are exposed to a continues water flow. Swimming activity is measured using a flow-through well plate in which 72 hpf to 120 hpf zebrafish embryos and larvae are exposed to a continues water flow. Swimming activity is measured using a low cost pipetting robot to investigate the influence of the medium exchange frequency on the toxicity of substances in the fish embryo toxicity assay (FET) method. The second bio-unit is a custom made pipetting robot which is equipped with a multi-pipette that automatically exposed zebrafish embryos aged 8 hpf to 48 hpf in a multi well plate. First experiments were conducted aiming to test both tools regarding their functionality and sensitivity.

WE002
Using a low cost pipetting robot to investigate the influence of the medium exchange frequency on the toxicity of substances in the fish embryo toxicity assay (FET)
S. Steffens, RWTH Aachen University, Institute for Environmental Research / Institute of for Environmental Research; L. Nuesser, RWTH Aachen University;
induction, and (4) embryotoxicity for brown trout and rainbow trout.

WE004 Use of in-vitro bioassays to characterize the environmental quality of sediments from the Adriatic and the Black Sea


Due to their geographic proximity, changes in the natural and human environment in the Adriatic and Black Sea represent a shared concern for Croatia, Italy, Montenegro, Romania, and Ukraine. The Interaction of industrial waste, human and animal activities, and environmental changes have contributed to a high level of organic contamination. In the Adriatic Sea, the contamination has been so severe that it has forced the closure of fishing activities in some areas. In order to improve marine ecosystem health, reliable methods to assess the environmental quality of sediments are needed. The aim of this study was to characterize the environmental quality of marine sediments from the littoral areas of the Adriatic Sea using in-vitro bioassays. The results of this study will be used to support decision-making processes related to marine environmental quality.

WE005 The Biotransformation Potential of Zebrafish Embryos

A. Tierbach, Eawag; K. Groh, Eawag Swiss Federal Institute of Aquatic Science and Technology; K. Schirmer, Eawag / Environmental Toxicology; M. Suter, Eawag

Zebrafish (Danio rerio) are an excellent model organism for the study of biotransformation and detoxification processes in aquatic environments. The aim of this study was to investigate the biotransformation potential of zebrafish embryos using in-vitro bioassays. The results of this study will be used to support decision-making processes related to marine environmental quality.

WE006 Performance of the avian acute oral guideline (OECD 223) in delivering desired endpoints and reducing the number of test subjects.


The avian oral guideline (OECD 223) is widely used to test the acute oral toxicity of chemicals in birds. However, the guideline has been criticized for its high number of test subjects and the fact that it does not always deliver the desired endpoints. The aim of this study was to evaluate the performance of the avian acute oral guideline (OECD 223) in delivering desired endpoints and reducing the number of test subjects. The results of this study will be used to support decision-making processes related to marine environmental quality.
concerns as well as animal welfare benefits. In this poster, we evaluate results from 186 recent studies with qual performed in 5 laboratories: 104 studies according to the US EPA guideline and 82 studies to the OECD 223 guideline. Approximately 70% of the studies included in the evaluation had an LD50 of >2000 mg/kg. Of the 1763 control birds used in all the evaluated studies, there were only 2 control mortalities (0.11%). Furthermore, 78% of all mortalities recorded in all the studies contained occurred within the first 3 of the test. These data can be used to confirm the assumptions and utility of the validation simulation, and provide an empirical basis for comparison of the observed performance of the test design, particularly with regard to the previously mentioned factors and animal use.

WE007 Development of high-throughput screening method for single and multi-generational developmental and reproductive toxicity of chemicals using the nematode Caenorhabditis elegans

H. Kim, M. Kim, J. Kim, University of Seoul; J. Choi, School of Environmental Engineering Graduate School of Energy and Environmental system Engineering

The EU Regulation on chemicals and their safe use has been made a provision to minimize the number of animal tests required in implementing the REACH (Registration, Evaluation, Authorization and Restriction of Chemical substances) regulation. Large-scale US research programs (e.g. ToxCast, Tox21) also outlined the need for non-mammalian toxicological models to test the potential health effects of a large number of chemicals while also reducing the use of traditional animal models. In this study, we developed high-throughput screening (HTS) method for developmental and reproductive toxicity of chemicals using the nematode Caenorhabditis elegans. The screening methods were optimized for the measurement on a single, as well as, on multi-generational effects of chemicals. C. elegans is an attractive alternative model because of its well characterized and evolutionarily conserved biology, low cost, and ability to be used in HTS. For developmental toxicity screening, C. elegans were exposed to chemicals from age 3 hours and up to 96 hours and H2O was measured at 12 hrs of time interval using COPAS Select, whereas for reproductive toxicity, C. elegans were exposed to chemicals from young adults for 72 hrs and their offsprings were counted. Method for multi-generational effects of chemicals was also optimized. To validate the optimized methods, the predictive value of the C. elegans was assessed against mammalian in vivo reproductive toxicity data. 12 chemicals having endpoints indicative of reproductive toxicity were selected from US EPA Toxicological Reference Database (ToxRefDB) as positive controls, while 8 chemicals that do not have such indications were used as negative controls. We found significant reduction on the number of offspring in C. elegans exposed to positive control chemicals, while no effect by negative control chemicals. Overall results support COPAS-based C. elegans developmental and reproductive toxicity screening test possess prediction power for mammalian reproduction toxicity hence has a considerable potential for development of first-tier HTS of reproduction toxicity. As Korean-REACH will come into force in 2015, we are conducting the experiments against chemicals on K-REACH registration list. Acknowledgement: This work was supported by the grant from the Korea Ministry of Environment as "Environmental Health R&D Program" (2012001130009).

WE008 Biotransformation of chemicals in 3D-hepatic fish spheroids

M. Baron, School of Biological Sciences; K. Mintram, Plymouth University / Safety Health Environment; A.N. Jha, Plymouth University / Safety, Health & Environmental Graduate School of Energy and Environmental system Engineering

The use of fish in vitro models for the bio-accumulation assessment of environmental pollutants is beginning to receive wider attention, partly due to increased pressure from regulators to replace, or at least reduce, the number of animal tests required in implementing the REACH (Registration, Evaluation, Authorization and Restriction of Chemical substances) regulation. Large-scale US research programs (e.g. ToxCast, Tox21) also outlined the need for non-mammalian toxicological models to test the potential health effects of a large number of chemicals while also reducing the use of traditional animal models. In this study, we developed high-throughput screening (HTS) method for developmental and reproductive toxicity of chemicals using the nematode Caenorhabditis elegans. The screening methods were optimized for the measurement on a single, as well as, on multi-generational effects of chemicals. C. elegans is an attractive alternative model because of its well characterized and evolutionarily conserved biology, low cost, and ability to be used in HTS. For developmental toxicity screening, C. elegans were exposed to chemicals from age 3 hours and up to 96 hours and H2O was measured at 12 hrs of time interval using COPAS Select, whereas for reproductive toxicity, C. elegans were exposed to chemicals from young adults for 72 hrs and their offsprings were counted. Method for multi-generational effects of chemicals was also optimized. To validate the optimized methods, the predictive value of the C. elegans was assessed against mammalian in vivo reproductive toxicity data. 12 chemicals having endpoints indicative of reproductive toxicity were selected from US EPA Toxicological Reference Database (ToxRefDB) as positive controls, while 8 chemicals that do not have such indications were used as negative controls. We found significant reduction on the number of offspring in C. elegans exposed to positive control chemicals, while no effect by negative control chemicals. Overall results support COPAS-based C. elegans developmental and reproductive toxicity screening test possess prediction power for mammalian reproduction toxicity hence has a considerable potential for development of first-tier HTS of reproduction toxicity. As Korean-REACH will come into force in 2015, we are conducting the experiments against chemicals on K-REACH registration list. Acknowledgement: This work was supported by the grant from the Korea Ministry of Environment as "Environmental Health R&D Program" (2012001130009).

WE009 Biosensor parameters optimization for rapid identification of environmental pollution

K. Mintram, R. Licbinsky, Transport Research Centre; B. Sebestova, J. Krejci, BVT Technologies

Chemical analysis and toxicity tests on living organisms are used for monitoring of individual environmental component quality. For toxicity tests, plants and animals of different trophic levels are used. These were selected due to their sensitivity to chemical compounds and environmental pollution. The advantage of bioassay is capability to total toxic analysis of substances of all trophic levels. Their disadvantage is particularly time consuming, because of a few days cultivation of organism with toxic substance or environmental samples. And time plays significant role in case of unexpected accidents because you need to know quickly, if sample is dangerous for the environment or not. New device is based on the principle of the biosensor and allows practical and rapid indication of environmental contamination. This biosensor uses the green algae that are very sensitive and are also used in the standard test described in ISO 8692. The principle consists of measurement of life cycle of algae. The life cycle is monitored by oxygen production after algae illumination. If the toxicity occurs, the oxygen production decreases. Measurement conditions were optimized for temperature, the ratio of light and darkness and the selection of suitable organism in order to achieve a short measuring time with sufficient sensitivity. The green algae Scenedesmus quadricauda and Pseudokirchneriella subcapitata were chosen for measurement. Measurements were made both on the selected chemical compounds and natural environmental samples of runoff waters and soil water leachates. Results of single measurement by this device were obtained within one hour, approximately 100 times faster than in the case of the 72-hour test according to 8692.

WE010 Scientific approaches to support the waiving of chronic fish tests

A. Kienzler, European Commission - Joint Research Centre / IHC System Toxicology Unit EURL ECVM; M. Halder, European Commission Joint Research Centre / DG Joint Research Centre IHC System Toxicology Unit EURL ECVM; A. Worth, European Commission Joint Research Centre / IHC Systems Toxicology Unit and EURL ECVM

Regulatory aquatic risk assessment schemes require toxicity testing of chemicals on a limited number of laboratory species; thus, extrapolation from the obtained toxic responses to all species representing that trophic level in the environment is a fundamental tenet of regulatory ecotoxicological risk assessment. To derive the PNEC for aquatic toxicity, safety factors are applied to the laboratory data. These factors are intended to account for interspecies differences in sensitivity, extrapolation from acute to chronic effects, the physicochemical complexity of natural water versus laboratory test media, and the complexity of the ecosystem versus single species laboratory tests. The choice of the factor (10, 100 or 1000) depends on the quality and quantity of the available data. EU chemicals policy (industrial chemicals, biocides, etc) encourages the use of all available information for hazard and risk assessment before new tests on vertebrates are proposed or conducted. EURL ECVM is currently exploring whether interspecies extrapolations and acute-to-chronic relationships can be used for supporting the waiving of chronic fish tests. For this purpose, we have extracted data (LC50, NOEC, for Daphnia magna and fish from various databases (US EPA Ecotox, Aquatic Japan MoE, Aquatic ECOTOC, Aquatic OASIS and ECHA CHEM). We are analysing: 1) Daphnia (acute and chronic) to fish chronic toxicity relationships, and 2) fish acute to chronic toxicity relationships, both on the whole dataset and classified by their mode of action (MOA). Our preliminary results suggest that when fish acute toxicity data are available they could be used to predict fish chronic toxicity; however, if fish acute data are not available, then Daphnia data could be used, as long as the predictive accuracy for different MOAs is kept in mind. Our findings show the potential of data-based approaches. Keywords: REACH, waiving of tests on vertebrates, interspecies correlations, acute-to-chronic relationships Disclamer: The opinions expressed herein are those of the authors and do not necessarily reflect the official views of the European Commission

WE011 Size DOES matter: determination of the critical molecular size for the uptake across the chorion of zebrafish (Danio rerio) embryos

K.E. Schneider, University of Heidelberg / Aquatic Ecology and Toxicology; K. Heyer, K. Kais, A. Keck, B. Sapel, University of Heidelberg / Centre for Organismal Studies; K.E. Schneider, University of Heidelberg / Centre for Organismal Studies

The chorion surrounds the unhatched fish embryo and has repeatedly been discussed as a potential barrier for the uptake of chemical substances. Since in July 2013 the new guideline 236 “Fish Embryo Acute Toxicity (FET) Test” for testing chemicals has been approved by the OECD, more detailed information on uptake mechanisms and a possible critical molecular size of chemicals for the uptake across the chorion is required. For this purpose, zebrafish embryos were exposed to high concentrations of a hydrophilic non-toxic polymer, polyethylene glycol (PEG). The molecular weight of the 6 different PEGs ranged from 2000 to 12000 D and were used as a parameter for the respective size. High concentrations of a
solute, which might not be able to pass the chorion, should evoke effects similar to osmosis. As a result, the chorion should shrink due to water efflux and restricted reflux during the equalization process, serving as an endpoint to determine a critical molecular size of PEGs to cross the chorion. To gain more information about the time-dependence of the movement across the chorion, two different steps were investigated: (1) the time-course of water efflux out of the perivitelline space sufficient to transfer to the high PEG concentration; (2) the equilibrium process until the chorion has reached minimal shape. Within 5 minutes after transfer into the solutions, the chorion of zebranow eggs showed strong shrinkage due to extreme water efflux unrespective of PEG. The extent of shrinkage could be shown to depend on (a) the solute concentration, (b) the molecular weight and (c) the duration of exposure. All eggs exposed to 9.76 mmol/L showed a stronger degree and shrinkage of chorionic eggs. Exposed to 3.75 mmol/L, while only 17% of eggs showed a minimal change. For PEG 2000 the chorion fully recovers within 5 hours after start of exposure, for PEG 3000 the chorion still shows minor changes after 24 hours. For PEGs ≥ 4000 D, the chorion showed size-dependent, heavy deformations of the chorion after 24 hours. Therefore, a reflux of water and PEG molecules into the chorion and a resulting re-shaping of the chorion could only be observed for eggs exposed to PEGs ≤ 4000 D. This indicates a barrier function of the chorion for molecules from a size between 3000 and 4000 D.

WE012 Extrapolation of aquatic hazard information to soil and sediment: do we capture all the risks?
C. Boegi, BASF Aktiengesellschaft; M. Lammi, ExxonMobil Biomedical Sciences, Inc.
Within a regulatory context, environmental risk assessments typically rely on aquatic ecotoxicity data only. However, whether or not aquatic hazard information of a substance sufficiently captures hazards towards other environmental compartments as well, remains a point of discussion. Under REACH for instance, the performance of environmental risk assessments is triggered by its environmental classification. As the current classification system only covers aquatic hazards, this is leading to cautious approaches as to when and how environmental risk assessments are undertaken. For example, the discussion on the scope of Exposure Assessment under REACH could not be settled due to the ongoing debate on whether the existing classification for aquatic hazards captures the risks to organisms in the soil and sediment compartments. This poster will summarise the findings of an ECETOC Task Force on the matter. A database of substances for which aquatic, soil and sediment ecotoxicity data are simultaneously available, has been established. In the current phase, the performance of equilibrium partitioning (EqP) theory to extrapolate aquatic hazard information to the soil and sediment compartments – an approach typically used with regulatory frameworks such as REACH – is assessed for different trophic levels. The accuracy of EqP in predicting toxicity towards soil and sediment organisms is discussed in light of different physical-chemical parameters, substance mode of action and other parameters. Finally, the discussion will cover cases where the existing aquatic data is sufficient to capture hazards/risks for all the compartments, and on the opposite, where it is not. Some proposals to remedy these insufficiencies will be made.

WE013 Development of an enzyme-linked immunosorbent assay (ELISA) for assessing vitellogenin content in the skin mucus of Perciformes (Teleostei: Acanthopterygii)
B. Allner, GOBI GmbH; M. Hennies, Tecomedical Development; M. Willner, IBACON GmbH; T. Schmidt, Hochschule Fresenius; L. Barche, P. Stahlshmidt-Allner, Gobiob GmbH
Vitellogenin (VG) is usually synthesized in females under the control of estrogenic disruption of zooplankton and fish early life stages. A sensitive and reliable ELISA has been developed to measure VG induction in the skin mucus of Perciformes with a non-destructive and simple sampling technique, with tremendous potential for novel approaches in toxicological assessment of estrogens.

WE014 Global data requirements for vertebrate testing of pesticides: Opportunities for best practice and harmonisation
n. burden, NCSRks; S.K. Maynard, Syngenta / Environmental Safety; L. Weltje, BASF SE / Agricultural Centre; M. Fryer, CRD HSE; J.R. Wheeler, Dow AgroSciences
Most new pesticide active substances and their products are developed for global use. Consequently, environmental data packages are developed to meet all the data requirements of the regions and countries for which registration is intended. These different geographic areas often have varying needs, requiring different and sometimes different endpoints for testing. Such requirements can greatly increase the number of vertebrate animals used without necessarily increasing the quality or utility of information for decision making. Here we review the global vertebrate data requirements for the major regions. This analysis is used to inform: (a) Recommendations for best practice to reduce the overall numbers of animals within current requirements, and (b) Highlight priorities for better global harmonisation. Such approaches could contribute to the development of short- and long-term strategies to reduce vertebrate animal testing for the global registration of pesticides, without compromising environmental protection.

WE015 PBT Assessment of Personal Care Products
S. Cassani, University of Insbruck / DISTA; A. Sangion, DISTA; F. Marzetta, University of Insbruck / QSAR Res Unit Environ Chem Ecotoxic Department; M. Hennies, Tecomedical Development; M. Willner, IBACON GmbH; M. Hennies, Tecomedical Development; M. Willner, IBACON GmbH; T. Schmidt, Hochschule Fresenius; L. Barche, P. Stahlshmidt-Allner, Gobiob GmbH
During the last years, several organic ingredients in Personal Care Products (PCPs) became compounds of increasing environmental concern, mainly because they are commonly detected in receiving waters. PCPs are widely used all over the world in order to obtain benefits in everyday life and to improve the quality of several products. However, there is limited understanding of how these emerging contaminants may affect aquatic wildlife communities. Some of these PCPs contain also endocrine disrupting compounds, and many of their other adverse effects are essentially unknown. In fact, data on persistence, bioaccumulation and toxicity are lacking and their environmental behavior need to be further investigated. Moreover, the determination of all the dangerous properties, required by REACH and Cos-me-te-ics Directive (Council Directive 76/768/EEC), is a long and difficult task and thus it is very important to have tools to quickly highlight the most hazardous compounds, focusing the experiments only on the prioritized compounds. Quantitative Structure–Activity Relationships (QSAR) models can help to generate data for the unknown activities and properties necessary to prioritize chemicals. Using QSR models, in this study we have screened the potential cumulative PBT (peristence, bioaccumulation, toxicity) behavior, the persistence and the (eco)toxicity of hundreds of PCPs. More than 500 chemicals such as flavor and fragrance agents, hair dyes ingredients, parabens, phthalates and sunscreen agents have been screened with different models and tools. The PBT assessment has been carried out with the Insbruck PBT Index and the US-EPA PBT Profiler. The PCPs identified as PBTs in agreement by the two methods have been specifically evaluated for their persistence by the Global Half-Life Index (GHLI) model included in Q SARINS and for toxicity by a specific QSR model, developed ad-hoc for personal care products, for the prediction of acute toxicity in Pimephales promelas. Particular attention has been devoted to the study of Applicability Domain of our models. Concluding, the potential environmental hazard of a big number of PCPs ingredients has been studied assessing their potential PBT behavior, persistence and fish toxicity. A priority list is therefore proposed, including the potentially most hazardous personal care products ingredients.

WE016 Use of a holistic approach in validation: QSR models versus experimental data
P. Bicheler, F. BAUER, KREATiS; F. Sahigara, P. Thomas, CEHTA SAS
There is a strong and recognised relationship between fugacity, chemical activity and potency for narcotic substances (MoA 1 and 2). High Accuracy Quantitative Structure Activity Relationships models (HA-QSARs) have been developed such that these thermodynamic laws are embedded in the algorithms. Thus using a cascade approach each endpoint (eg. daphnid toxicity) providing results as good as those from quality data necessary to prioritize chemicals. Using QSR models, in this study we have screened the potential cumulative PBT (peristence, bioaccumulation, toxicity) behavior, the persistence and the (eco)toxicity of hundreds of PCPs. More than 500 chemicals such as flavor and fragrance agents, hair dyes ingredients, parabens, phthalates and sunscreen agents have been screened with different models and tools. The PBT assessment has been carried out with the Insbruck PBT Index and the US-EPA PBT Profiler. The PCPs identified as PB Ts in agreement by the two methods have been specifically evaluated for their persistence by the Global Half-Life Index (GHLI) model included in Q SARINS and for toxicity by a specific QSR model, developed ad-hoc for personal care products, for the prediction of acute toxicity in Pimephales promelas. Particular attention has been devoted to the study of Applicability Domain of our models. Concluding, the potential environmental hazard of a big number of PCPs ingredients has been studied assessing their potential PBT behavior, persistence and fish toxicity. A priority list is therefore proposed, including the potentially most hazardous personal care products ingredients.

WE017 Development and Validation of an in vivo (in situ) assay to measure Solubility of organic contaminants in water
R. Haines, NERC UK Centre for Environmental Data Valuation; F. Sahigara, P. Thomas, CEHTA SAS
Quantitative Structure–Activity Relationships models (QSARs) have been developed such that these thermodynamic laws are embedded in the algorithms. Thus using a cascade approach each endpoint (eg. daphnid toxicity) providing results as good as those from quality data necessary to prioritize chemicals. Using QSR models, in this study we have screened the potential cumulative PBT (peristence, bioaccumulation, toxicity) behavior, the persistence and the (eco)toxicity of hundreds of PCPs. More than 500 chemicals such as flavor and fragrance agents, hair dyes ingredients, parabens, phthalates and sunscreen agents have been screened with different models and tools. The PBT assessment has been carried out with the Insbruck PBT Index and the US-EPA PBT Profiler. The PCPs identified as PB Ts in agreement by the two methods have been specifically evaluated for their persistence by the Global Half-Life Index (GHLI) model included in Q SARINS and for toxicity by a specific QSR model, developed ad-hoc for personal care products, for the prediction of acute toxicity in Pimephales promelas. Particular attention has been devoted to the study of Applicability Domain of our models. Concluding, the potential environmental hazard of a big number of PCPs ingredients has been studied assessing their potential PBT behavior, persistence and fish toxicity. A priority list is therefore proposed, including the potentially most hazardous personal care products ingredients.

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WE017
High Accuracy QSARs for the prediction of acute and chronic aquatic toxicity  
T. P. Thomas, CEHTRA SAS, P. Bicherei, KREATIS, F. Sahigara, CEHTRA SAS

The thermodynamic relationship between the chemical activity and the toxicity of narcotic chemicals has recently been hypothesised by Mackay et al in 2009. In 2013, an ECETOC task force carried out an extensive project on a large existing dataset to validate Mackay’s hypothesis that the chemical activity can be used to accurately predict the effects of narcotic species. The task force tentatively proposed a relationship for acute and chronic effects for MoA1 substances and this was recently made available as ECETOC Technical Report 120. Using these data as a starting point, KREATIS extensively reworked these tentative algorithms and created the first High Accuracy QSARs (HA-QSARs) with R²>0.95 for acute aquatic toxicity to Fish, Daphnia and Algae. Once the acute toxicity QSAR results had been demonstrated, it was decided to test at least as accurate as experimental data for MoA1 substances, the work was then extended to MoA2 substances to see if similar levels of accuracy could be obtained. Further work was also initiated on the chronic toxicity endpoints for MoA1 substances (where metabolism and analytical issues could significantly influence the experimental results and therefore accuracy in predictions). Future developments including the understanding of Acute to Chronic Ratios (ACR) is possible to adjust the high narcotic emissions, aiming improved toxicity; QSAR References: Mackay D. Arnot J. Petkova E. Wallace K. Call D. Brooke L. Veith G. 2009. The physicochemical basis of QSARs for baseline toxicity. SAR and QSAR in Environmental Research 20:3, 393-414. ECETOC Technical Report No. 120, 2013. Activity-Based Relationships for Aquatic Ecotoxicology Data: Use of the Activity Approach to Strengthen MoA Predictions. iSAFERAT® (In Silico Algorithm For Environment Risk Assessment and Toxicity). 2014. iSAFERAT® v.1.1.

WE018
Automatic counter and characterization in ecotoxicology assays  
S. Arcuri, Université de l’Avignon / Dep Biology; CESAM; M. Oliveira e Silva, R.E. Martins, Université d’Aveiro / DETI; IEETA; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM

One of the most widespread bioassays used in ecotoxicology to evaluate effluents and contaminated waters is performed with Daphnia, where most of the acute tests require a repetitive and time consuming counting procedure, aiming endpoints such as mortality and reproduction capability. In this study we present a new technology for counting organisms embedded in a solution (e.g. Daphnia in ecotoxicology assays) based on organism serialisation and conduction along a tubular cell passing through an electronic optical detector. The generated analogue optical signal is then processed by a computational system to perform the organism counting and, eventually, a chromatatic classification (to infer its motibility/mortality). With this technique it is possible to adjust the light chromatic emission, aiming improved organism/solution contrast for counting purposes or, alternatively, to filter away the characteristic green colour of the organism algae food (enhancing the quality of the chromatatic classification of the organism). This technology allows a real-time counting of organisms present in a solution with a fully automated procedure, thus avoiding current error-prone “manual” counting and operator fatigue; thus, enabling data handling. It significantly reduces global procedural time in ecotoxicology. Chromatatic classification of organisms may also allow better data results as it will give objective indication of each organism motibility/mortality state. It is also possible to simultaneously process several solutions in a multi-counter realization of this apparatus.

WE019
RTgill-W1 cell line assay to test for acute toxicity of effluents  
M. Knöbel, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; K. Schirmer, Eawag / Environmental Toxicology

We established the rainbow trout (Oncorhyncus mykiss) gill cell line, RTgill-W1, as a fast and inexpensive means to predict fish acute toxicity for organic industrial chemicals. The outcome of the cell line assay compared very well not only to the fish acute toxicity test results but also to results obtained in the zebrafish (Danio rerio) embryo toxicity test, which has meanwhile been accepted as the OECD test guideline 213. The success of the RTgill-W1 cell line assay has led to a CEFIC-LRI/UK-NC3Rs-supported round-robin study to bring this assay closer to the in vivo situation. This study is currently addressing this issue as well as fish and fish acute toxicity test. One question raised in this context was if the RTgill-W1 cell line assay can also be used to predict fish acute toxicity of effluents. Assessing effluents with such an animal-free method would be highly desirable: it is estimated that in North America alone, about three millions of fish are sacrificed annually for whole effluent testing. In fact, several previous studies have indicated that, indeed, the RTgill-W1 cell line is suitable for such an application. For example, Dayeh, Schirmer and Bols (2002, Water Research, 36: 3727-3738) introduced a protocol with which they tested whole-water effluents from a paper mill over the course of one year in both the RTgill-W1 cells and the fish acute toxicity test using rainbow trout. Out of sixteen samples assessed, only one was toxic to rainbow trout and this was also the one causing cytotoxicity in RTgill-W1 cells. In light of this and recent requests to use the RTgill-W1 cell line for whole water effluent testing, we have set-up the method in which salts of the tissue culture medium, L-15/ex, are added to pre-filtered raw effluent samples to establish isotonic conditions for direct cell exposure. Inasmuch as effluents samples nowadays are rarely acutely toxic, we spiked the effluent samples with 3,4-dichloroaniline as reference compound, demonstrating that the cell line can detect acute toxicity from even very low concentrations. We are currently exploiting this method to test effluents of different origin and compare the effects observed in the cells also with those elicited by the same samples in zebrafish embryos [ISO 15088:2007(E)]. Demonstrating the ability of the RTgill-W1 cell line assay to sensitively respond to raw effluents of different origin will be an attractive complement to assessing single chemicals for acute toxicity.

WE020
Cyto and genotoxicity of two industrial wastes on coelomocytes of Eisenia fetida  
S. Curtese, Université Côte d’Azur / Eawag, Swiss Federal Institute of Aquatic Science and Technology / CONICET; A. Alberdi, UNLU; W.D. Di Martino, Universidad Nacional de Lujan/CONICET

The management of industrial wastes is an important economic and environmental problem. The presence of pollutants in industrial waste can produce genotoxic activity in several species. Thus, evaluation of the toxic and genotoxic potential of industrial wastes towards organisms is acquiring a particular significance. The earthworm Eisenia fetida is model specie in toxicity studies. Single-cell eletrical and optical microscopy (SCOE); is a sensitive method for detecting DNA damage in the coelomocytes of earthworms. Coelomocytes were exposed to in vivo and ex situ conditions to determine the genotoxicity of two industrial wastes. The industrial wastes correspond to foundry sand, related to metallicurgical activity, and sludge produced by cosmetic industry. The objective of present work is evaluate cyto and genotoxic potential on coelomocytes of earthworms exposed to in vivo or ex situ conditions, using two in vivo endpoints, as a whole or a subpopulation of coelomocytes, respectively. For the ex situ exposures the coelomocytes were exposed to aqueous leachate of each waste during 1 h. For the ex situ exposures the earthworms were exposed to solids wastes during 7 and 14 days. The cytotoxicity was determined by Trypan blue dye. The SCOE was performed to assess the genotoxicity of aqueous leachate and solid wastes. The results shows solid wastes and its aqueous leachate increase levels of DNA damage. The study indicate the importance of implementing into environmental monitoring genotoxicity test together classical physicochemical analysis for regulate the final disposal of industrial wastes.

WE021
Genotoxic potential of insecticides inudacloprid evaluated at different test organisms  

The indiscriminate use of pesticides has become a serious environmental concern. Being one of them, the inudacloprid (IMI) is one of the most widely used world wide. In Brazil, it is used in sugarcane, citrus, cotton, and coffee crops. During 2009, in São Paulo, Brasil, 1,934 tons of IMI were applied, mainly reducing global pesticide use. The genotoxic potential of the insecticide IMI in the aquatic environment is unknown. Using the Daphnia magna and Drosophila melanogaster model organism and a new in vivo ex situ methodology, we investigated whether the aquatic ecotoxicological profile of IMI could be used to estimate its potential environmental impact. We investigated the potential ecological effects of IMI as well as its possible ecological effects. However, few studies have examined the toxicity at the genetic level. This is one of the biggest challenges for the scientific community, who is concerned about the impacts of these contaminants on the environment and human health. The use of live organisms (biocenators) can reveal the presence of stressors from environmental pollutants and allow the use for monitoring their negative effects. Among them, the plants and the amphibian animal are considered as excellent genetic models to detect genotoxic effects. Several studies have examined the toxicity of IMI as well as its possible ecological effects. However, few studies have examined the toxicity at the genetic level. This is one of the biggest challenges for the scientific community, who is concerned about the impacts of these contaminants on the environment and human health. The use of live organisms (biocenators) can reveal the presence of stressors from environmental pollutants and allow the use for monitoring their negative effects. Among them, the plants and the amphibian animal are considered as excellent genetic models to detect genotoxic effects. In this study, we evaluated the effects of IMI on the genetic material in the plants Allium cepa and Tradescantia pallida and in the fish Oreochromis niloticus following exposure to different concentrations of this insecticide. Allium cepa seeds, inflorescences of T. pallida and O. niloticus erythrocytes were used. The results in A. cepa assay and T. pallida micronucleus test showed that a dose-dependent change in the number of MN was observed, which increased the frequency of MN. The results in O. niloticus erythrocytes for comet assay demonstrated induction the primary damage on the DNA by increasing the frequency of strand breaks and alkali labile sites in the concentrations tested. The results from the MN and other nuclear alterations (NA) test in O. niloticus erythrocytes indicates the IMI did not cause a significant increase in MN frequency and if of some NA as blebbed nuclei (BL) e notched nuclei (NT) in the higher concentration used. Summarizing IMI was genotoxic in plants and induced primary DNA damage but no damage at the chromosome level in O. niloticus erythrocytes. These factors should be taken into account when applying this pesticide.
WE022 Characterisation of the in vitro RTgutGC spheroid model: suitability as an ecotoxicology tool?

L.M. Langan, Biological Sciences; S.F. Owen, AstraZeneca / Safety Health Environment; S. Jackson, W. Purcell, Plymouth University / School of Biomedical Sciences; A.N. Iha, Plymouth University / Biological Sciences

The bioactivation of toxicants is the physiological route by which chemicals are metabolized into reactive components. Little is known about the evaluation of in vitro toxicity should be performed on cell derived systems which have adequate and relevant metabolic activity. Adding to this, the model should represent a relevant route of exposure. The gastrointestinal tract is the primary route of exposure to environmental toxins located in food chains and the rainbow trout gastrointestinal delivered cell RTgutGC has been suggested as a potentially suitable model for in vitro toxicity testing. However, its metabolic activity in terms of ability to take up and detoxify specific compounds has not been characterised. 2D cell culture systems were used and exposed to environmentally relevant concentrations of copper over a 72h exposure, and compared to a previous in vitro study. Metabolic activity in the gut cell line was quantified using EROD and epoxide hydrolase (EH) activity as indicators of the cell line’s capacity for xenobiotic metabolism of both drugs and P AH’s. GST activity was investigated to identify whether the cell line was capable of phase II metabolism, in both monolayer and spheroid forms. Initial results indicate that both the spheroid form and monolayer form of the cell line is metabolically active for both CYP and EH, with the spheroid form showing higher relevant activity. Copper exposure in the spheroids shows an increase over time as expected, with a similar response noted in the inserts. There was no significant differences in the recorded copper concentrations in the fluid/media of the spheroid culture and the insert culture potentially indicating a similar metabolic uptake of the metal. This will be investigated further.

WE023 Toxicity of the endocrine disruptor nonylphenol in plant development at environmental concentrations

M. Catala Rodriguez, P. Morales, Universidad Rey Juan Carlos / Biology and Geology Physics and Inorganic Chemistry; S. Esteban, Universidad Rey Juan Carlos / Area de Medicina Preventiva y Salud Publica; H. Cortés, Universidad Rey Juan Carlos / Biology and Geology Physics and Inorganic Chemistry; Y. Valcácel, Universidad Rey Juan Carlos / Department of PreventiveMedicine Public Health Immunology and MedicalMicrobiology

In recent years there is a growing concern among the scientific community about the presence of the so-called emergent pollutants in waters of different countries, especially those that have the ability to alter the hormonal system (endocrine-disrupting compounds). One of the substances found almost ubiquitously and in higher concentrations is the alkylphenol nonylphenol. The aim of this work is to assess the acute and sub-chronic toxicity of environmental concentrations of nonylphenol in riparian vascular plant development using spores of the fern Polystichum setiferum and a biomarker-based approach: mitochondrial activity (biomarker of cell viability), chlorophyll (plant physiology) and DNA content (growth). Mitochondrial activity and DNA content show that nonylphenol induces acute and sub-chronic toxicity at 48 h and a week. Significant effects are observed in both biomarkers in fern spores at ng/L but chlorophyll autofluorescence shows little changes. The inhibition of germination by natural allelochemicals has been reported to be related with the active hydroxyl group of phenolic compounds and largely independent of the structural nucleus to which it is attached. Results presented in this study indicate the environmental concentrations of nonylphenol could interfere with higher plant germination development by mimicking natural allelochemicals likely posing ecophysiological risks.

WE024 Semi-automated detection of goitrogenic compounds using transgenic zebrafish embryos and the VAST Biolimag platform

S. Jarque, Masaryk University / Faculty of Science RECETOX; E. Fetter, Department of Bioanalytical Ecotoxicology; M. Pipal, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; M. Smutna, Masaryk University Faculty of Science RECETOX; L. Blaha, Masaryk University, Faculty of Science / Faculty of Science RECETOX; S. Scholz, Helmholtz Centre for Environmental Research / Department of Bioanalytical Ecotoxicology

The thyroid metabolism plays a crucial role in various metabolic, behavioral and developmental processes such as growth and maturation. However, there is very little knowledge on environmentally relevant pollutants that may interfere with these developmental pathways. Gene expression changes of thyroid hormone synthesizing genes can be used as an endpoint to detect thyroid disruption. In order to facilitate an easy and fast detection of gene expression alterations in zebrafish embryos, we used the transgenic zebrafish line tg(tg:mCherry) combined with the VAST (Vertebrate Automated Screening Technology) Biolimag platform. While the strain tg(tg:mCherry) reflects the expression of thyroglobulin gene encoding the thyroid hormone precursor, the VAST system allows an easier and relatively fast handling of zebrafish embryos compared to manual procedures. The tandem was shown as efficient for the screening and detection of compounds with goitrogenic activity, as it is disclosed by the dose-response increases of fluorescence signal in fish embryos after exposure to a number of prototypical model thyroid endocrine disrupters such as ethynylethioeurea, sulfamethoxazole, potassium perchlorate, phenol glucuronid, resorcinol and 6-propyl-2-thiouracil. The present approach may be also applied for the assessment of potential thyroid disrupting activities associated to environmental samples. For example, preliminary data suggest positive responses in complexes extract of cyanobacteria and their toxins (microcystins), which represent a major environmental problem during nutrient pollution induced by freshwater algae blooms.

WE025 Endocrine-disrupting potentials of equine estrogens in the medaka Oryzias latipes: in silico and DNA microarray studies

K. Arzoni, Prefectural University of Kumamoto / Faculty of Env Symbiotic Science; M. Uchida, Mizukibiotec; H. Ishibashi, Shokei University Junior College / Department of Food and Nutrition; Y. Ishibashi, Prefectural University of Kumamoto / Faculty of Environmental and Symbiotic Science

Although several previous studies have demonstrated the presence of equine estrogens in the aquatic environment, limited data are currently available on the endocrine-disrupting potentials in fish and the risks they pose to aquatic organisms. To investigate the interactions of major equine estrogens equilin (Eq) and equilenin (Eqn), as well as their metabolites 17α-dihydroequilenin, 17β-dihydroequilenin, and 17α-dihydroequilin, with the estrogen receptor α (ERα) of medaka (Oryzias latipes), a three-dimensional model of the ligand-binding domain (LBD) of ERα was built in silico, and docking simulations were performed. The docking simulation showed that the interaction of 17β-dihydroequilenin with the ERα LBD is the most potent, followed by those of 17α-dihydroequilenin and 17β-dihydroequilin, whereas those of Eq and Eqn were least potent. We further analyzed gene expression profiles in the livers of male medaka exposed to Eq and Eqn. A DNA microarray representing 6000 genes revealed that 24-h exposure to Eq and Eqn (100 nM) upregulated the expression of 6 and 34 genes in the livers of males, respectively. Genes upregulated by Eq included the estrogenic biomarker genes vitellogenin and choriogenins, suggesting the estrogenic potential of Eq. In contrast, Eqn exposure upregulated several cancer-related genes, such as mediator complex subunit 16 and RAS oncogene family members, suggesting a carcinogenic potential for Eqn. These results suggest that equine estrogens may have not only endocrine-disrupting potentials via the ERα signaling pathway but also carcinogenic potency in male medaka.

WE026 Proposal for a sub-letality test using zebrafish embryo A. Wigh, UMR CNRS LEHNA; S. Bony, Université de Lyon; A. Devaux, Université de Lyon-ENTR & Observatoire Géologie Physics and Ino

Respecting the principles of the 3 R’s (reduce, refine, replace), the FET test, which uses the zebrafish (Danio rerio), was basically designed as an alternative to the fish acute toxicity test to determine acute toxicity of chemicals or environmental samples on embryonic stages. In the FET, only lethal endpoints are used to calculate a LC50 value after 96h of exposure. The design of the test easily allows to recur performed. The docking simulation analysis indicated that this test works well to integrate these parameters using a new calculation method resulting in a so called “FET index” (iFET). This complementary approach is proposed for the assessment of sub-acute hazards, particularly relevant to evaluate a chronic toxicity risk for low and/or multi contaminated environmental samples. The method is first tested with three different chemicals 3,4-dichloroaniline (3,4-DCA), methoxy-methanesulphonate (MMS) and cadmium, as well as two WWTP effluents (a Biologically Treated Effluent (BTE) and the same followed by ozonation (BTE+O3). In samples studied here, LC50 was not calculable. Thus, the percentage of affected embryos based on both FET and iFET is given and the classic FET scores are compared with the iFET scores through colour grades. As a negative control, ISO water results in very similar scores with both systems. In the 3,4-DCA sample (3.7 ng/L), the average mortality (44%) results in a medium risk scoring at the end of the test. When applying the iFET calculation, this percentage rises up to 71.33% and characterizes an excessive risk. A low risk is observed with the iFET in the WWTP samples BTE and BTE+O3 (conc. from 6.875 to 100% and 27.5 to 100%, respectively) whereas some of these samples are considered without risk using the classic FET with very little attention on ethylene glycol toxicity risk. So the extended FET enables to detect toxicity with a higher sensitivity, due to the possibility to take into account sub-letal effects earlier than embryo’s death. Since such effects can potentially reduce zebrafish fitness by affecting growth, survival and reproduction, this extended FET offers interesting perspectives in aquatic ecotoxicology.

WE027 DAMIER - A research project for the development and application of high accuracy in-silico models for REACH

P. Thomas, CEHTRA SAS; P. Bicheler, KREATIS; P. ROUSSEAUX, H. OSUNA, J. DELAFOSSE, Processium; S. Kent, Roulantain Research Company / Brixham Environmental Laboratory; S. BETAT, N. DELPIT, Laboratoires des Pyrénées et des Landes; c. vuillet, ISA

Despite recommendations by the European Chemicals Agency (ECHA) to use in-silico approaches as an alternative to replace animal testing, the majority of existing QSAR models have been designed for screening purposes and are therefore...
considered unacceptable to replace experimental studies due to lack of accuracy and strict validation. This was the basis for proposing DAMIER (stands for Développement et applications de modèles informatiques pour REACH) research project which was funded in 2014 under the 17th French FUI (Fonds Unique Interministériel) call for projects. The objective of the DAMIER project is to replace current experimental studies needed for chemicals registration under REACH regulation using computational models of physiological and toxicological properties. This work will involve the development of High Accuracy Quantitative Structure-Activity Relationship (HA-QSAR) models for numerous REACH annex VII and VIII endpoints. All the HA-QSARs developed under this project will be validated following the recommended five OECD principles for QSAR models1. This poster will provide an overview of the various research activities and the status of the development of three mammalian cell lines over the three years amongst all the five project partners (Two SMES, KREATIS (co-ordinator) and Processium; the Institut des Sciences Analytiques (ISA); the Laboratoires des Pyrénées et des Landes (LPL) and Rovaltain Research Company). A workflow will be provided to get a better understanding of the collaboration within the project partners, in particular how the laboratory-based work will be carried out throughout the project work to support the development and demonstrate the validation of DAMIER HA-QSARs, (for instance, procurement of data from new, innovative and reliable experimental methods, some of which generated by DAMIER). Finally, an overview of the validation strategy of these models will be provided to make them suitable for registration purposes under REACH. Keywords: DAMIER; QSAR; in-silico; REACH References: OECD principles for the validation, for regulatory purposes, of quantitative structure-activity relationship (QSAR) studies, 2006; OECD Principles for the validation of in vitro QSAR studies, 2007; OECD Principles for the validation of in silico QSAR studies, 2010; Damier Final Report 2014-2016; DAMIER project partners.

WE028
Generation of reactive oxygen species in a fish cell line and two mammalian cell lines after exposure to acid mine water

O. Hellbusch1,2, M. M. Malherbe1, C. J. du Toit1,2, D. W. King1,2, R. G. Coetzee1,2, J. F. du Preez1,2, D. F. van der Merwe1,2,2.1 Department of Paraclinical Science; 2 Department of Pharmacology, University of Pretoria, South Africa

Acid mine water (AMD) is released from abandoned mines, and can have a serious impact on freshwater systems. The effects of AMD are mainly associated with the generation of reactive oxygen species (ROS), which is implicated as a pathway of toxicity in aquatic animals exposed to AMD. The cell culture method is a reliable and robust method for the generation of ROS in vivo. Reactive oxygen species (ROS) production and resultant oxidative stress (OS) has been implicated as a pathway of toxicity in aquatic animals exposed to AMD. The fathead minnow embryo as a model for the development of alternative testing methods in ecotoxicology. After the adoption of the fish embryo toxicity test (FET) with WE031 as the official OECD test guideline 236, it seems essential to adapt the fathead minnow embryo (larvae) was subjected to real-time reverse transcription PCR (RT-qPCR) for all diagnostic genes. Then the results were compared to those obtained using adult fish (liver transcriptomics), enabling us to discuss the potential replacement of juvenile/adult fish testing by embryo tests.

WE030
Applicability of the mode of action concept for sensitivity categorization of aquatic ecotoxicological data

M. Duhonho ITM1 / Chemical Risk Assessment; W. Drost, National Institute for Occupational Safety and Health,痊

The fathead minnow embryo (Pimephales promelas) is one of the three most common OECD test species together with the zebrafish (Danio rerio) and the Japanese medaka (Oryzias latipes) for ecotoxicological testing. After the adoption of the fish embryo toxicity test (FET) with WE031 as the official OECD test guideline 236, it seems essential to adapt the fish embryo toxicity test protocol to other OECD species to broaden the applicability of the test. We aimed to validate the applicability of the mode of action concept and to avoid animal experiments (Art. 13, 25, the REACH Regulation (EC 1907/2006) provides several options to avoid the chronic fish test. 240 substances from the OECD eChemPortal and ICS database of the German Federal Environment Agency have been analysed for sensitivity differences between Daphnia and fish to evaluate whether and when chronic fish tests can be avoided without underestimating environmental hazard. Only studies conducted according to the European Union Technical Guidance Documents were considered. The result showed that species sensitivity in chronic testing is associated with sensitivity during acute testing. A categorization system for acute sensitivity comparison was proposed that may support the integrated testing strategy. The aim of this study was the evaluation of the mode of action (MoA) concept according to Verhaar to investigate refinement options for chemicals and to check the applicability for substances that exert a specific mode of action by interacting with certain receptors (e.g. pesticides). MoA assignments were made using OECD tool box. Substances allocated to MoA3 appeared to be more sensitive to fish in acute and chronic testing whereas substances allocated to MoA1 and MoA2 were in average slightly more sensitive to Daphnia. However, overall the evaluation suggested that a distinct species sensitivity is probably not associated with a specific MoA class. The results further suggested that the acute sensitivity comparison system can be applied independent of the MoA class and is also applicable for substances allocated to MoA4. Furthermore, acute to chronic ratios were derived for fish and Daphnia data for each MoA class.

WE031
The fathead minnow embryo as a model for the development of alternative testing methods in ecotoxicology

S. Bohler, COS Centre for Organismal Studies; S. Oberhauser, University of Heidelberg / COS Centre for Organismal Studies; T. Braunbeck, University of Heidelberg / Centre for Organismal Studies

The fathead minnow embryo (Pimephales promelas) is one of the three most common OECD test species together with the zebrafish (Danio rerio) and the Japanese medaka (Oryzias latipes) for ecotoxicological testing. After the adoption of the fish embryo toxicity test (FET) with WE031 as the official OECD test guideline 236, it seems essential to adapt the fish embryo toxicity test protocol to other OECD species to broaden the applicability of the test. Prior to standardisation, it is important to clearly identify the developmental stages of the fathead minnow embryo and beyond by combining technology and morphological and histological which are of toxicological relevance. Therefore, the primary purpose of this study was to provide a detailed overview of fathead minnow normal development. In order to elucidate pathological variability in fathead minnow development, the adapted fathead minnow FET was carried out for the known teratogens isoniazid, ethanol, caffeine and hydroxyurea; in addition to acute and subchronic effects in embryo toxicity data for teratogenesis were recorded. Since part of the tested teratogens are known to provoke skeletal abnormalities, the development of cartilaginous and bony elements in the head region of the fathead minnow embryo was studied by means of specific histological analyses and compared to the normal development of the fathead minnow skeleton. For this end, fish exposed to the test...
chemicals were stained to identify malformations of cartilage and bone in the head region of the fathead minnow embryo after termination of the FET. Malformations were recorded and compared to existing zebrafish data. Keywords: ecotoxicology, fathead minnow embryo, FET, teratogenicity

WE032 Development and characterisation of the first immortalised humpback whale cell line
M. Burkard, Griffith University / Southern Ocean Persistent Organic Pollution Program; D. Whitworth, The University of Queensland / School of Veterinary Science; K. Schirmer, Eawag / Environmental Toxicology; S.M. Bengtson Nash, Griffith University / Southern Ocean Persistent Organic Pollutants Program (SOPOP).

Polar foraging humpback whales (Megaptera novaeangliae) are dependent on a lipid-rich diet and accumulated lipid stores to undertake the longest migration and associated period of voluntary fasting, known in any mammal. This extreme life-history leads to an elevated risk of Persistent Organic Pollutants (POPs) accumulation and toxicity. Measuring the toxicological impact of POPs on wild populations of humpbacks is a greater challenge in chemical risk assessment. New and stable methods are required to study humpback whale species-specific sensitivity and cellular responses. Herein, we report for the first time the immortalisation of humpback whale cell lines. Primary fibroblasts were derived from dermal biopsies using a primary explant method. Cells of the second passage were transfected with a plasmid encoding the simian virus (SV40) large T antigen and in lieu of conditions and additional equipment. With the advent of applying such transfected and cell lines were continuously subcultured with hygromycin selection. In obtained cell lines, the presence of the introduced plasmid was verified by PCR. The cell lines have been propagated eight times and showed positive immunoreaction to mouse anti-SV40T. This indicates stable introduction of the plasmid into the chromosomal DNA. Cloned cell lines will be further characterized including cloning of telomerase activity. The telomerase activity is known to non-immortalised fibroblasts of different passages will reveal the normal telomere length and its temporal shortening dynamics. Overall, we present research toward a novel infinite cell line to provide more accurate and reproducible approaches towards chemical risk assessments of southern hemisphere humpback whales.

WE033 Ready-to-use recombinant yeast assays for in-field detection of endocrine disruptors
M. Bitter, Masaryk University, Fac. of Science / Faculty of Science RECETOX; S. Jarque, Masaryk University / Faculty of Science RECETOX; K. Hilscherova, Masaryk University, Faculty of Science, RECETOX / RECETOX Research Centre for Toxic Compounds in the Environment

Recombinant yeast assays (RYAs) constitute a suitable tool for the environmental monitoring of compounds with endocrine disrupting activities, notably estrogenicity and androgenicity. Conventional procedures require yeast reconstitution from frozen stock, which usually takes several days and demands specialized conditions and additional equipment. With the advent of applying such transfected and cell lines were continuously subcultured with hygromycin selection. In obtained cell lines, the presence of the introduced plasmid was verified by PCR. The cell lines have been propagated eight times and showed positive immunoreaction to mouse anti-SV40T. This indicates stable introduction of the plasmid into the chromosomal DNA. Cloned cell lines will be further characterized including cloning of telomerase activity. The telomerase activity is known to non-immortalised fibroblasts of different passages will reveal the normal telomere length and its temporal shortening dynamics. Overall, we present research toward a novel infinite cell line to provide more accurate and reproducible approaches towards chemical risk assessments of southern hemisphere humpback whales.

WE034 Visualisation of metabolic activities using an integrated living embryo microarray and Fluorescence Ratiometric Imaging (FRIM) system
F. Zhu, RMIT University / School of Applied Sciences; D. Baker, Vancouver Island University / Institute for Stem Cell Sciences & Technologies; Griffith University / School of Applied Sciences; M. Sewell, University of Auckland / School of Biological Sciences; D. Wiikowski, RMIT University / School of Applied Sciences

Significant progress in the development of physico-optical oxygen sensors using luminescence quenching by molecular oxygen has recently been made. Sensing such microsensors is, however, still performed in small glass chambers that hold single specimens and thus not amenable for high-throughtput data acquisition. Moreover, conventional measurements can only be taken along one dimension and provide classical, averaged results of metabolic activities of an entire specimen. As such they do not provide any spatial-temporal information of, for example, 2D and 2D+ time oxygen distributions inside the living tissues or medium surrounding the specimen. These profound analytical limitations restrict fundamental studies on how abiotic and pharmacological stimuli affect embryonic physiology, the developmental stage when animals are typically considered to be most sensitive to environmental perturbations. These drawbacks can, however, be experimentally addressed by an emerging field of microfluidic Lab-on-a-Chip (LOC) technologies combined with sophisticated photomicroscopic sensors. A Lab-on-a-Chip represents a new direction that may complement and revolutionise research on toxicology and physiology in vivo. In this work, we present a proof-of-concept approach by using microfluidic Lab-on-a-Chip (LOC) technologies combined with the latest generation of sensor foil oxygen detection system. Our prototype device is capable of immobilising live zebrafish embryos with continuous flow perfusion, while the sensor uses innovative Fluorescence Ratiometric Imaging (FRIM) technology that can kinetically quantify the temporal patterns of aqueous oxygen gradients at a very fine scale based on signals coming from an optical sensor referred to as a sensor foil. Our preliminary objective was to determine whether the sensor foil oxygen detection system can be effectively integrated into the chip-based platform and allow for characterization of oxygen gradients with the accuracy and precision needed to estimate embryonic oxygen consumption rates in zebrafish embryos. We demonstrate that by embedding the sensor foils onto the microfluidic living embryo array system, we were able to perform in situ FRIM on developing zebrafish embryos. Future integration of microfluidic chip-based technologies with FRIM technology represents a noteworthy direction to miniaturise and revolutionise research on metabolism and physiology in vivo.

WE035 Alternative Methods and Study Design Refinements to Reduce or Replace Animal Use in Wildlife Toxicity Tests
B.A. Rattner, USGS-Patuxent Wildlife Research Ctr / USGS

The development of new and refinement of existing “alternative testing” is a regulatory imperative, but the regulatory example is the tiered testing protocol for the evaluation of candidate non-toxic shot used in hunting in the United States (Federal Register 62:63608-63615, 1998). Using extant toxicity data, several candidate shot materials (metals, alloys, polymers) have been approved for use without any animal testing whatsoever. From a research perspective, data from domestic birds and laboratory rodents have been employed to design and conduct small-scale sequential dosing studies to assess toxicity and risk of metals (e.g., vanadium, J Toxicol Environ Health 69A:331-351, 2006), pharmaceuticals (e.g., diclofenac, Environ Toxicol Chem 27:2341-2345, 2008) and pesticides (e.g., diphacinone, Environ Toxicol Chem 30:1213-1222, 2011) in wild birds. Findings from these and other studies have documented remarkable differences in sensitivity among avian species. This presentation will review and highlight some of the challenges in making inter-specific extrapolations, modifying wildlife toxicity tests to reduce animal use, and employing these data to assess risk.

WE036 Fish-gut-on-chip: Development of ultrathin support for intestinal cell culture from rainbow trout
C. Drieschner, Griffith University / Southern Ocean Persistent Organics Program; M. Minghetti, Eawag / Environmental Toxicology; P. Renaud, EPF Lausanne School of Architecture Civil and Environmental Engineering; K. Schirmer, Eawag / Environmental Toxicology

Transwells (permeable membrane inserts for cell culture) are one of the most used systems to study epithelial barrier functions in vitro. The porous membrane allows permeability assays, microscopy and transepithelial electrical resistance measurement (TEER). However, relatively high membrane thickness (25 µm, cell height ~10 µm) and low porosity (< 1 %) can limit full development and sensitive measurement of epithelial barrier properties. In this study we report on the use of newly developed supports for cell culture. Precisely, ultrathin membranes (1 µm) were composed of aluminum oxide. They feature a porosity of 15% and show enhanced permeability for small molecules such as Lucifer yellow, Dextran 40 and bovine serum albumin. Moreover, we applied impedance spectroscopy, a non-invasive and lable-free bio-sensing tool, to examine the electrical properties of epithelial cells during the formation of a monolayer. Herein, we used the epithelial-like cell line RTgutGC, derived from the hindgut of Rainbow trout, to recreate the intestinal barrier of fish in vitro. Our results suggest alumina membranes as biocompatible substrates for cell culture. The ultrathin membrane, which is realized in a silicon-chip, can be integrated in transwells and in the future in microfluidic bioreactors. For transwell cultures we observe polarization of the epithelial cells after 2-3 weeks using confocal microscopy. Impedance spectroscopy indicates tightening of the epithelial monolayer over time by the formation of tight junctions. Additionally, impedance spectroscopy provides information on cell viability, which facilitates the evaluation of toxicant impacts on barrier properties. To summarize, with the newly developed support for cell culture we can overcome some of the shortcomings of conventional cell culture membranes (low permeability, thickness) and allow for more sensitive measurements (microscopy, impedance spectroscopy).
Immortalized human cells: current approach in organ toxicity testing

R. Grillari, University of Natural Resources and Life Sciences Vienna; J. Grillari, G. Manhart, I. Lämmermann, BOKU University Vienna; M. Uhl, M. Paparella, Unweltbundesamt Environment Agency Austria; M. FURHAKER, WAU FZJ. For the protection of emerging containers in integrated testing strategy (ITS) with application of in-vitro biological tools in the context of adverse outcome pathways (AOPs) is discussed for the protection of human and environmental health. However, currently used in vitro test systems show several limitations. Whereas, normal human somatic cells, albeit expressing cell type specific markers and functions, show a limited proliferative capacity, human tumor tissue derived cells in many instances do not express features of the corresponding normal counterpart cells and thus do not represent the in vivo situation accurately. The aim of this study was to evaluate the potential of highly differentiated, continuously growing human cells from the endothelium (HUVEC/TERT7) as well as kidney tissue (RPTEC/TERT1) for ITS. In a first approach, the cellular response to chromate treatment was tested using conventional MIT and apoptosis assay as well as induction of DNA damage using Comet assay or γ-HAX staining. Additionally, detection of changes of the cell type specific phenotype or of essential cell cycle regulators and heat-shock proteins upon treatment with chromate was envisaged. Moreover, the uptake of chromium into the cells was tested using ICP-MS (total Cr) and a photometric method (CrVI). As a result, both cell lines reproducibly show cytototoxic effects of these substances at IC50 values of 7.4 μM and 6.5 μM for dichromate. This is in line with the measured estrogenicity after exposure to the selected compounds. Interestingly, both cell lines show a clear increase in γ-H2AX positive cells as indicator of DNA damage. The induction of heat shock proteins (Hsp27, Hsp70, and Hsp90) was cell line dependent and effects could be seen for concentrations as low as 0.5 μM of dichromate for RPTEC/TERT1 and 1 μM for HUVEC/TERT7 cell line. Whereas, no change in expression of cell type specific markers was observed in RPTEC/TERT1 cells, chronic treatment of HUVEC/TERT7 cells resulted in changes of the expression level of von Willebrand Factor, an endothelial cell type specific marker protein. Therefore, we have shown that human highly differentiated, standardizable cells line respond to stressors and might have a high potential as novel in vitro test systems for the detection of emerging contaminants in the near future.

Comparative vitellogenin expression in two alternative fish models using 17α-Ethinylestradiol

M. Baron, School of Biological Sciences; C. Dummett, University of Plymouth; T. J. Charman, Heriot-Watt University / School of Life Sciences; A.N. Jha, Plymouth University / Biological Sciences

The use of alternative testing strategies is currently receiving world-wide attention to reduce and ultimately, replace live fish testing. In particular, the zebrafish (Danio rerio) is increasingly being used as an alternative model with which to measure in vitro effects. However, the use of zebrafish is limited to the study of endogenous or slowly acting xenobiotics. Therefore, we have compared two different immortalized standard cell types, the immortalized HUVEC/TERT7 cell line and the immortalized human liver spheroids (i.e. 18S rRNA for spheroids and Occhocypinus mysis) 3-D liver spheroid (in vitro) and zebrafish larvae (in vivo) model, after exposure to the endogenous estrogen 17α-Ethinylestradiol (EE2). Following optimisation of RNA extraction and reverse transcription for both spheroids and zebrafish larvae, time-course exposures to a range of EE2 concentrations (0.01 – 1 µg/L) were applied, and vg gene expression measured using qPCR. Suitable housekeeping genes (i.e. 18S rRNA for spheroids and β-actin for the larvae) for each system were fully validated. Our results suggests that 3-D liver spheroids may be a sensitive model with which to measure vg expression after exposure to endogenous estrogens and compares well with data generated from zebrafish larvae exposures. Keywords: 3-D liver spheroids, zebrafish, vitellogenin, qPCR

Pharmaceuticals and fish: effects in alternative in vitro cellular and embryonal models

M. Michelenka, Masaryk University, RECETOX / Faculty of Science RECETOX; M. Pipl, Masaryk University; A. Jonas, Masaryk University, RECETOX / Faculty of Science; M. Tran, University of Hradec Králové; P. Huška, Masaryk University, Faculty of Science / Faculty of Science RECETOX

Pharmaceuticals are major micropollutants in water environment and represent a significant contribution to the endogenous estrogen 17β-estradiol (E2) pool in aquatic organisms. They are environmentally transported along the food web and accumulate in organisms in higher trophic levels. Therefore, there is an ongoing effort to develop and assess alternative testing systems that can be used to assess the ecotoxicological impact of pharmaceuticals. Aquatic mammalian species are less sensitive to pharmaceuticals due to their lower bioavailability and metabolism. However, alternative in vitro systems are needed for testing pharmaceuticals on fish species. Therefore, the aim of this study was to compare the effects of pharmaceuticals on non-mammalian fish species in vitro. We have compared two alternative in vitro systems: the zebrafish larvae (Danio rerio) and liver spheroid (3T3) model. The results indicate that both systems are suitable for testing pharmaceuticals and that human highly differentiated, standardizable cells line respond to stressors and might have a high potential as novel in vitro test systems for the detection of emerging contaminants in the near future.

Prediction and screening of fish biotransformation half-lives

E. Papa, Department of Theoretical and Applied Sciences; L. van der Wal, REACH Mastery; J.A. Arnot, ARC Arnot Research & Consulting / Department of Physical Environmental Science; A. Sangion, DIStA; S. Cassani, P. Gramatica, University of Insubria / DIStA

The bioaccumulation assessment of new and existing chemicals is a legal requirement for many regulatory agencies and a relevant step in exposure and risk assessment. Biotransformation half-lives (HLs) derived from in vitro studies are used in the mathematical models used in compliance with the “OECD properties for the validation, for regulatory purpose, of (Q)SAR Models”. In addition, and in order to improve models accuracy, a combinatorial approach was applied to predictions generated by both the new QSARs and the EPI Suite HLs model. Results show an increase in the percentage of accurate predictions from ~85% for single models, to ~94% for the combined model. Finally, predictions by different models were combined by Principal Component Analysis (PCA) to screen the biotransformation half-lives of an additional dataset of over 1300 chemicals already screened as potential PBTs. Results from the PCA show that the potential, predicted, PBT behaviour of compounds may be overestimated and therefore the potential for biotransformation could be considered to refine PBT assessment procedures. This study is an example of how alternative methods to animal testing, i.e. in silico QSAR models, can be successfully used at the screening level in hazard and risk assessment procedures, not only to fill data gaps but also to address uncertainty and refine previous assessments.

Investigation of anti-estrogenic substances as disrupters of embryonic gonadal development in Gallus gallus domesticus

L. Jessl, R. Lenz, F. Massing, Goethe University Frankfurt am Main / Aquatic Ecotoxicology; J. Oehlmann, Johann Wolfgang Goethe-Universität Frankfurt / Aquatic Ecotoxicology

Avian eggs have been shown to be suitable test systems for toxicological assessment of substances. The embryo develops outside the mother whereby changes in development are well observable. One major advantage of the chick embryo as an experimental subject is that in contrast to the recognized rodent assays no parental animals have to be killed. In addition chicken embryos dissected before hatching are not rated as animal experiments and are therefore considered as an in vitro system. The endocrine system of birds is largely identical to that of mammals and avian eggs are known to be sensitive to a number of estrogenic and anti-estrogenic disrupting chemicals. Therefore the Federal Ministry of Education and Research supports the development of an animal replacement system for the assessment of the hormon-toxic potential of chemicals as disrupters of embryonic gonadal development. So the present study aims to develop a replacement method for testing hormonally active environmental chemicals on the basis of chicken eggs. Results from a study not presented here show that the effect of an in silico estrogen and tamoxifen at doses between 0.1 and 10 μg/g egg on embryonic gonadal development of Gallus gallus domesticus are presented. Although fluoridestan did not affect sexual differentiation of male and female embryos directly, the feminization of genetic males by the potent estrogen EE2, however, was completely reversed by EE2 treatment. Therefore, we have shown that human highly differentiated, standardizable cells line respond to stressors and might have a high potential as novel in vitro test systems for the detection of emerging contaminants in the near future.

We have shown that human highly differentiated, standardizable cells line respond to stressors and might have a high potential as novel in vitro test systems for the detection of emerging contaminants in the near future.
µg/g egg resulted in a significant size reduction of the left ovary by up to 50% compared with the control group. In general it can be shown that the pure anti-estrogen fulvestrant does not influence sex differentiation in developing chicken embryos while the partial anti-estrogen tamoxifen impairs the differentiation of the gonads. Due to the largely identical hormone system of birds and mammals, an impairment of human sex differentiation cannot be excluded. [11] Lange, Hartel, Meyer. 2002. Evolution of oestrogen functions in vertebrates. J Steroid Biochem Mol Biol. 83:219-226.

Invertebrate models in environmental toxicology (P)

WE042
GammarusChip - customised and automated microperfusion technology for marine ecotoxicity biotests
R. Cartlidge, RMIT; D. Nugegoda, D. Wlodkowski, RMIT University / School of Applied Sciences

Traditional marine ecotoxicity testing is inherently labor intensive, requiring extensive manual procedures to be performed both to set up the tests and more importantly to collect experimental readouts. Moreover, static test procedures offer poor control of water parameters such as toxicant concentration and dissolved oxygen, which affect toxicity and are important considerations in evaluating environmental impacts of aquatic pollution. So far only minimal levels of automation have been adopted in ecotoxicology. Our current work attempts to address the current limitations by capitalizing on latest advances in microfluidics, 3D printing and laser micromachining technologies to develop highly customized, low cost and high-throughput devices. This will for the first time enable automation of ecotoxicity biotests performed on marine test organisms. This work describes an investigation into applications of microfluidic Lab-on-a-Chip technology for highly customised flow through toxicity testing chambers in marine ecotoxicity. We present development of a proof-of-concept laboratory automation system to perform ecotoxicity tests on a native marine amphipod Allorchestes compressa. Our innovative system incorporates a flow through design that enables the biotests to be run in a closed or open loop circuits. Automated fluidic control technology allows for concentrations of toxicants to be spiked and diluted using computerized manifold of miniaturized pumps and valves. Miniaturized video cameras monitor the amphipods during the experiment and innovative video analysis algorithms provide us with real time sub-lethal endpoints such as changes in swimming activity that would otherwise go unnoticed. A key advantage of this flow through system as compared to conventional approach is the automation of amphipods and environment for sub-lethal behavioral endpoints. The GammarusChip technology is inexpensive, portable, easy to use and require only a small amount of bench space to be operated. Overall waste effluent required is significantly reduced when compared to static renewal testing as well as the time needed to set-up and maintain the experiments.

WE043
Automation of Artoxkit-M ecotoxicity biotest using a microfluidic Lab-on-a-Chip technology
Y. Huang, RMIT University / School of Applied Sciences; C.C. Aldasoro, City University London / School of Engineering and Mathematical Sciences; G.A. Persson, MicroBioTests Inc; D. Wlodkowski, RMIT University / School of Applied Sciences

Despite its critical importance, the broad deployment of high-throughput whole-organism ecotoxicity tests is profoundly limited by the lack of appropriate bio-compatible automation, integrated optoelectronic sensors, and the associated electronics and analysis algorithms. These major shortcomings have not been yet addressed, and as a result all such biotests are still performed manually, are time consuming, laborious and not available for high-throughput analysis. In this work, we present development of a miniaturized Lab-on-a-Chip (LOC) platform for automation of acute ecotoxicity test based on a marine crustacean Artemia franciscana (Artoxkit M™). We for the first time demonstrate development of a proof-of-concept technology for rapid assessment of sub-lethal, behavioral ecotoxicological impacts. The system working principles are as follows. The GammarusChip technology incorporates a time-resolved video data analysis to dynamically assess impact of the reference toxicant on swimming behavior of marine crustacean Artemia franciscana. Our system design combined: (i) innovative LOC flow-through device for on-chip keeping of Artemia sp.; (ii) automated mechatronic hardware for LOC fluidic system actuation and data acquisition; and (iii) innovative video analysis algorithms for the segmentation, tracking, visualization and movement analysis. The algorithm are written in Matlab and released open-source at the website www.phageisight.org. The chip-based device supported rapid loading and keeping of free swimming crustacean larvae suspended in a continuous microfluidic perfusion as a mean of toxin delivery in both open-loop and closed-loop modalities. This system was also capable of performing fully programmable time-lapse and video-microscopy of multiple samples for rapid ecotoxicity analysis. Custom video algorithms were then developed for scoring dynamic features of video frames, capturing detailed statistical information on test specimen movements. This enabled development of a new test protocol to dynamically detect sub-lethal behavioral end-points based on a swimming behavior of Artemia franciscana. This work provides a foundation for automated high throughput ecotoxicity biotests using innovative Lab-on-a-Chip technologies. A key advantage of this flow through system as compared to conventional static testing is the miniaturization and automation of analysis and special emphasis on sub-lethal behavioral parameters.

WE044
Toxic effects of Chaetoceros calcitrans extracts on the embryos of three commercially relevant shellfish species
G. Giménez Papilo; A. Buer, Rostock University; M. Miller, Plant and Food; T. Harwood, H. Kaspar, Cawthron Institute

Chaetoceros calcitrans is a marine diatom extensively used as food in aquaculture production facilities. It is considered nutritionally good for shellfish embryos development due to its high content in polyunsaturated fatty acids (PUFA; Miller et al, 2003), but some authors have shown that it is able to produce polyunsaturated aldehydes (PUA; Ragg et al 2010) which have deleterious effects on marine invertebrate reproduction (Wichard et al 2007) and induce apoptosis in invertebrate embryos and cell cultures from different phyla (Romano et al 2003, Adolph et al 2004). The standardized shellfish embryo-based ecotoxicological assay (ASTM 2004) has been adapted and used as screening tool for the detection of C. calcitrans toxicity during its culture growth. The most toxic sample, most likely to contain PUA, was analyzed by GC-MS. Greenshell mussel™ (Perna canaliculus), blue mussel (Mytilus galloprovincialis) and Pacific oyster (Crassostrea gigas) were exposed to the same aqueous extracts of C. calcitrans samples and the yield of D-larvae after 48h was recorded and compared to each control group. The extracts did not present any toxic effect on M. galloprovincialis embryos (>80% D-larvae), one extract was toxic for C. gigas embryos (<60% D-larvae), sampling each extract led to the further extracts were toxic for P. canaliculus embryos (< 30% D-larvae). The sample toxic to C. gigas and P. canaliculus corresponded to the pellet of a 3-day old culture of C. calcitrans, but no PUA was detected by GC-MS. The toxicity of C. calcitrans on shellfish embryos development, mainly P. canaliculus, has been confirmed, but the compound(s) that causes the toxic effect still remain(s) unknown.

WE045
Investigation of toxicity pathway according to the change in concentration of chlorpyrifos and -oxen in Caenorhabditis elegans
J. Roh, Division of Environmental Science and Ecological Engineering / Division of Environmental Science and Ecological Engineering; J. Kwon, Korea University / Division of Environmental Science and Ecological Engineering

Chlorpyrifos (CP) is a well-known organophosphorus pesticide that produces a neurotoxic metabolite, chlorpyrifosoxon (CPO) that inhibits acetylcholinesterase (AChE) activity, by cytochrome P450 isozymes. Time-course changes in cellular processes including metabolic transformation and AChE activity are dependent upon the changes in the concentrations of CP and CPO in the body. The primary goals of this study were (1) to investigate the changes in metabolism-related gene expression and enzyme inhibition by CPO with exposure time and (2) to link these responses to residual CP and CPO concentrations in Caenorhabditis elegans under toxic conditions. Toxicology path: metabolism to AChE activity to toxicity to the expected environmental concentration. The risk quotient was...
compared to population-level impacts to determine how accurately it predicted effects at the population level. The potential hazard of this product to aquatic ecosystems will be discussed.

WE047 Quantifying sublethal effects of glyphosate and Roundup® to Daphnia magna using a free proteomic approach and enzyme activity assay and video tracking
P. Roslev, L.R. Hansen, M. Østergaard, Aalborg University / Biology and Environmental Science
Glyphosate (N-phosphonomethyl)glycine is the active ingredient in a range of popular broad-spectrum, non-selective herbicide formulations. The toxicity of this herbicide to non-target aquatic organisms such as *Daphnia magna* is often evaluated using conventional toxicity assays that focus on endpoints such as immobility and mortality. In this study, we investigated sublethal effects of glyphosate and Roundup® to *D. magna* using video tracking for quantifying behavioral changes, and a novel fluorescence based assay for measuring in vivo hydrolytic enzyme activity (FLEA assay). Roundup® exposure resulted in concentration-dependent inhibition of alkaline phosphatase activity in *D. magna*. The inhibition of alkaline phosphatase by Roundup® was temperature-dependent, with lowest inhibition at 14 °C and greater inhibition at 20 and 26 °C. Exposure of *D. magna* to sublethal concentrations of glyphosate resulted in behavioral effects quantified as decreases in average swimming velocity and distance moved whereas the inactive time in defined arenas increased. Exposure of *D. magna* to binary mixtures of glyphosate and copper (Cu) attenuated acute metal toxicity. The results suggest that glyphosate toxicity is caused by both glyphosate and metal toxicity, and that behavioral changes may be applied as a quantitative and sensitive tool for detecting sublethal effects of glyphosate and Roundup® to *D. magna*. The inhibitory effect of Roundup® on alkaline phosphatase in non-target organisms warrants further investigations.

WE048 Assessment of response of *Daphnia magna* exposed to contaminated sediments.
A.S. Sobrino-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia Laboratorio Alejandro Villalobos
In this study an evaluation of the health status of *Daphnia magna* was performed using 6 biomarkers to detect toxic and genotoxic effects of sediments contaminated with metals and persistent organic compounds, to prove the usefulness of these sensors in biomonitoring studies and ecological risk assessment. 14 sediment samples were assessed levels of metals and PAH, PCB and organochlorine pesticides (OP) and bioassays in parallel, were conducted with *Daphnia magna* nauplii to determine the toxicity of sediments. The effect on the following physiological parameters: Respiration rate, excretion rate, feed rate, Index O:N and 2 biomarkers: lipid peroxidation (MDA) and genetic damage (Comet Assay). The data from the physiological parameters and biomarkers were integrated to calculate the index BRI (Biomarker response index) to establish the state of health of the organisms exposed to sediment. The results showed that average levels of metals in sediments varied from 19.8 to 119.6 mg L⁻¹ and Cops (PAHs, PCBs, OP) form 0.01 to 827.13 mg g⁻¹. In multiple correlation analysis performed with data from BRI index and levels of pollutants, a significant relationship between the concentrations of Cd, Cr, Ni, Pb, V, PAHs, PCBs and the response of biomarkers evaluated was observed. Sample 1 had the lower levels of contaminants and BRI value was similar to the control group. The lowest values of BRI index that showed highest survival with severe alterations were obtained in tests with samples 3, 4 and 6. In these samples was observed that the concentrations of metals and Cops are higher than the values established in the Sediment Quality Tables (NOAA). (effects range-low causing adverse effects). From the above it is clear that the evaluated biomarkers provided information that matches with the levels of contaminants in sediments. Therefore the battery of biomarkers used could be a useful tool for studies of environmental biomonitring and ecological risk.

WE049 Relative expression of vitellogenin and vitellogenin-receptor genes in *Macrobrachium rosenbergii* exposed to chlordecone: an in-situ study case
A. Sobrino-Figueroa, R. Giommoni, Laboratory of Animal Ecology and Ecotoxicology; M. Hankeenne, University of Liege / Center for Protein Engineering; E. Boulangé-Lecomte, Laboratory of Ecotoxicology; J. Forget-Leray, University of Le Havre / Laboratory of Ecotoxicology; J. Thome, Liege University / Laboratory of Animal Ecology et Ecotoxicology
Assessing in environmental quality, effects of xenobiotic compounds (e.g. ingesting chemicals, pesticides) have an increased concern because they could interfere with endocrine system of organisms. Their effects are extensively described in vertebrates but less studied in invertebrates, while these organisms represent the major part of the aquatic biota. The endocrine system is mediated by hormones which regulate several physiological processes such as reproduction and juvenile growth. Endocrine disruptors could interfere at different stages of the signaling pathway of a hormone by several mechanisms and could thus induce adverse effects on endocrine system of organisms. In this in-situ study, we have investigated the effects of chlordecone, an organochlorinated pesticide, on the endocrine system of the crustacean decapod, *Macrobrachium rosenbergii*, by measuring relative expression of the vitellogenin gene in hepatopancreas of organisms coming from a control site and a contaminated site. In invertebrates, vitellogenin (Vtg) is the precursor of yolk proteins (e.g. vitellins) which supply energy reserves available during embryonic and larval development. Due to its crucial role, vitellogenin have been described as exposure biomarker for endocrine disruptors. In addition, we have evaluated the relative expression of the vitellogenin receptor (VtGr) gene in gonadal tissue. Males and females prawns of *Macrobrachium rosenbergii* were sampled in a control site and an aquaculture pond supply by a contaminated river (0.33 g L⁻¹ of chlordecone). Hepatopancreas and gonadal tissues were dissected and total RNA was extracted and analyzed by quantitative RT-PCR. The results revealed that Vtg and VtGr gene expressions were influenced by chlordecone exposure in *M.rosenbergii*. Indeed, we noticed an increase of both genes, in exposed prawns compared to control one, whatever the gender. This observation suggests that disruption of endocrine system could therefore occur following an exposure to an estrogenic compound. The prawns reproduction and the population dynamics could thus be indirectly impacted by the environmental presence of this compound. This study allows us to better understand the mechanisms of action of chlordecone and shows also that chlordecone could be an endocrine disruptor compound in crustaceans which could have some adverse effects on hormonal system of crustaceans and thus on populations and ecosystems.

WE050 PRELIMINARY DATA ON TOXICOKINETICS OF AN AZO DYE IN THE MARINE AMPHIPD PARHYALE HAWEIANSIS
A. dos Santos, M.C. Artal, University of Sao Paulo - USP / Toxicology and Toxicant Analysis; J.A. Wodzimierski, University of CAMPINAS, B. Silva, Sao Paulo State University - UNESP / Chemistry; M.V. zanoni, UNIVERSIDADE ESTADUAL PAULISTA - UNESP / QUIMIC A ANALITICA; G.d. Umbuzeiro, FACULTY OF TECHNOLOGY - UNICAMP / LEAL Parhyale haewaisis is a marine amphipod worldwide distributed that are starting to be used in ecotoxicology. They are easy to culture in the lab and measure around 8 to 12mm. Dye exposure used in this study were exposed to 10 different azo dyes in 10 concentrations. A pool of hemolymph from 10 organisms was diluted in ultrapure water and liquid-liquid extracted (2.5DCM:1MeOH). LC-MS/MS (Linear Ion Trap Quadrapole LC/MS/MS Mass Spectrometer) was employed for quantification of DR1. To verify if CYP and GST were involved in biotransformation of DR1 in freshwater organisms such as *Parhyale* No information was found about absorption and distribution of those compounds in microcrustacean aquatic organisms. Thus, the aim of this study was to obtain data on absorption and biotransformation of DR1 in *P. haewaisis*. Adult organisms of *P. haewaisis* were exposed to different non-lethal concentrations of commercial DR1 (nominal concentrations of 0.01 to 5 mg L⁻¹) using 4 and 12 hours of exposure and hemolymph was collected. A pool of hepatic homogenate from 10 organs was diluted in ultrapure water and liquid-liquid extracted (2.5DCM:1MeOH). LC-MS/MS (Linear Ion Trap Quadrapole LC/MS/MS Mass Spectrometer) was employed for quantification of DR1. To verify if CYP and GST were involved in DR1 metabolism in *P. haewaisis*, neotenes were exposed to 1 mg L⁻¹ with and without an enzymatic CYP inhibitor (0.1 mg L⁻¹) for 96 hours. GST activity was measured in Tribolium cochenillae (from Center for Toxicology, USDA) and DR1 was quantified in the hemolymph from < 0.001 to 0.281 mg L⁻¹ (LOQ=0.001 mg L⁻¹). The concentration of the dye in the hemolymph increased with concentration and time of exposure. We observed an increase in GST activity in DR1 exposed organisms, as compared to negative control. When CYP inhibitor was added GST activity was reduced to negative control levels. We conclude that DR1 is absorbed by *P. haewaisis* and it is possible to measure its concentration in hemolymph of such small organisms. We also suggest that CYP and GST are involved in DR1 biotransformation also in *P. haewaisis*. More experiments are being conducted with different times of exposure to understand the absorption behavior of DR1. Also metabolites of DR1 will be analyzed in hemolymph and in other tissues. Acknowledgement: CAPES and FAPESP Thematic Project 2008/10449-7.

WE051 Proteome variations in *Macrobrachium rosenbergii* exposed to chlordecone: a gel-free proteomic approach
A. Lafontaine, Laboratory of Animal Ecology and Ecotoxicology; G. Mazzucelli, Laboratoire de Biologie Marine et du Milieu Aquatique / Laboratory of Animal Ecology et Ecotoxicology; J. Forget-Leray, Univesity of Le Havre / Laboratory of Ecotoxicology; J. Thome, Liege University / Laboratory of Animal Ecology et Ecotoxicology
Some xenobiotic compounds (e.g. industrial chemicals, pesticides) are well known to interfere with endocrine system of organisms in various ways. Their effects are extensively described in vertebrates and less in invertebrates while these organisms represent the major part of the aquatic biota. Endocrine disruptors could interfere at different stages of the signaling pathway of a hormone by several mechanisms. They can modify the metabolism of circulating hormones, which regulate several physiological processes (e.g. reproduction, growth). Therefore, the endocrine system of organisms could be disrupted. During the last decades, proteomic approach could have been developed progressively in the field of ecotoxicology. Proteomics allow assessing changes in the expression of specific proteins and/or protein groups, occurring in response stress conditions, as for example chemical exposure. Proteomics could also allow elucidation of the underlying molecular pathways leading to adverse effects for organisms. In this study, we have
investigated the effects of chlordane, an organochlorinated pesticide, on the endocrine system of the crustacean decapod, *Macrobrachium rosenbergii* by measuring variations of protein expression in hepatopancreas tissue of organisms coming from a control tank and a contaminated tank using a “gel-free” proteomic analysis. The prawns of *M. rosenbergii* were exposed for one month to chlordane at three environmental relevant concentrations (0.2 µg.L$^{-1}$, 2 µg.L$^{-1}$, 20 µg.L$^{-1}$). The results revealed that the chlordane concentration of *M. rosenbergii* is linked to the chlordane exposure expression. Exposure of several proteins was significantly modulated between the different treatments. Most of these proteins are cytoskeletal proteins or proteins involved in energy or immune metabolism. But, other proteins involved in reproduction or development processes showed significant changes in their expression. Due to the fact that these proteins play an important role in the hormonal system of crustaceans, results suggest that chlordane is likely to be an endocrine disruptor for crustaceans. This study shows that chlordane is an endocrine disruptor in crustaceans which could have some adverse effects on hormonal system and thus on the populations and ecosystems. Results also highlight that proteomics is an appropriate way to better understand the mechanism of action of endocrine disruptor compounds and their impacts on population dynamics.

**WE052**

**Responses of antioxidant genes and survival in the crab *Macrobrachium japonicus* following to perfluorooctane sulfonate exposure**

K. Park, Department of Fisheries and Ocean Science; W. Kim, University of South Florida; C. Nes, caused by PFOS treatments. In terms of gene expression, we suggest analyzing parallel or binding short and/or long-term exposure using eco-toxicological approach.

**WE053**

**Addressing the recovery of feeding rates in post-exposure feeding bioassays: *Carcinus maenas* as a case study**

A. Pais-Costa, MARE Marine and Environmental Sciences Centre / Department of Life Sciences IMAR CMA; P. Acedo, Instituto de Investigacion en Recursos Cinegeticos / SaBio IREC; J. Marques, MARE Marine and Environmental Sciences Centre / Department of Life Sciences IMAR CMA; M. Martinez-Haro, IMAR-CMA (Marine and Environmental Research Centre / Department of Life Sciences)

Post-exposure bioassays are used in environmental assessment as a cost-effective tool, but the effects of organism's recovery after exposure to pollutant has not yet been addressed in detail. The objective of this research was therefore to determine the time period during which the feeding rate (FR) of a test species remains impaired after the exposure, i.e., when it can be used for post-exposure test (post-exposure time), in order to establish a safe window in which the FR endpoint signals the contact with the toxicant. This led us to evaluate the stability of the post-exposure FR response up to 24 hours after two different contaminant exposure periods (48 h and 96 h) for two subletal concentrations of cadmium (Cd [0.5 µg.L$^{-1}$ and 5 µg.L$^{-1}$], as a reference toxicant) in laboratory conditions. The experiments were performed using 18 crabs* Carcinus maenas* (48 h and 96 h exposure) and experimental groups were designed: one control group without exposure to Cd, plus two more for each of the Cd concentrations, one for each exposure period. Peeding tests were carried out at five different times in each experimental group: a) the control group feeding tests were performed immediately after starting the experiment (0 h) - the result from this test represented the baseline for all experimental groups, and at 48 h, 72 h, 96 h and 120 h after the start of the experiment; b) for animals exposed to Cd, the tests were carried out after the exposure period (EP), and at EP+3 h, EP+6 h, EP+12 h and EP+24 h (post-exposure times). The mean FR in the control group did not differ over time ($p > 0.05$). With regard to controls, mean FRs were statistically lower in the organisms exposed to 5 µg Cd/L for all the post-exposure times tested for both 48 h and 96 h exposure periods ($p < 0.05$). However, no statistical differences were detected for any of the organisms exposed to 0.5 mg Cd/L except for the post-exposure times EP+12 h and EP+24 h of the 96 h exposure period ($p < 0.05$). For the groups exposed to Cd, no significant differences between FR at EP and all the remaining post-exposure time intervals were detected at any of the exposure periods ($p < 0.05$). Although not significant, a slight increase on the feeding rate was detected for EP+6 h of the 48 h exposure period. Results showed that feeding depression was a stable endpoint up to 24 h after Cd exposure, which is useful for ecotoxicological bioassays.

**WE054**

**Cysteine proteinase and thioredoxin activities on egg and juveniles development of Gammarus pulex**

E. Gismondi, Laboratory of Animal Ecology and Ecotoxicology; J. Thome, C. Joaquim-Justo, Liege University / Laboratory of Animal Ecology et Ecotoxicology

Several pollutants are well known to cause endocrine disruption which could lead to modify some biological processes such as reproduction or the development of exposed organisms. However, the limited knowledge of the endocrine systems of invertebrates makes difficult the assessment of effects of xenobiotics considered as endocrine disruptors. The aim of this study was to compare the effects of two anti-androgens: cysteine proteinase (CPA, anti-androgenic steroid) and thioredoxin (anti-androgenic non-steroid) on Gammarus pulex. The parameters recorded were egg development (i.e. hatching time, neonate length), juvenile development (i.e. time of first mol, growth) and spermatopore number in males. Eggs were removed from the mother and bioassays are used in environmental assessment as a cost-effective tool, but the effects of organism’s recovery after exposure to pollutant has not yet been addressed in detail. The objective of this research was therefore to determine the time period during which the feeding rate (FR) of a test species remains impaired after the exposure, i.e., when it can be used for post-exposure test (post-exposure time), in order to establish a safe window in which the FR endpoint signals the contact with the toxicant. This led us to evaluate the stability of the post-exposure FR response up to 24 hours after two different contaminant exposure periods (48 h and 96 h) for two subletal concentrations of cadmium (Cd [0.5 µg.L$^{-1}$ and 5 µg.L$^{-1}$], as a reference toxicant) in laboratory conditions. The experiments were performed using 18 crabs* Carcinus maenas* (48 h and 96 h exposure) and experimental groups were designed: one control group without exposure to Cd, plus two more for each of the Cd concentrations, one for each exposure period. Peeding tests were carried out at five different times in each experimental group: a) the control group feeding tests were performed immediately after starting the experiment (0 h) - the result from this test represented the baseline for all experimental groups, and at 48 h, 72 h, 96 h and 120 h after the start of the experiment; b) for animals exposed to Cd, the tests were carried out after the exposure period (EP), and at EP+3 h, EP+6 h, EP+12 h and EP+24 h (post-exposure times). The mean FR in the control group did not differ over time ($p > 0.05$). With regard to controls, mean FRs were statistically lower in the organisms exposed to 5
WE058

Effects of biological and molecular gene responses on the abalone Haliotis discus hannai responding to temperature stress

K. Park, Department of Fisheries and Ocean Science; J. Lee, Chonnam National University / Department of Aquatic Life Medicine; J. Kang, Pukyong National University / Department of Aquatic Life Medicine; J. Kim, Gangwon Provincial College / Department of Marine Life Science and Aquaculture; I. Kwak, Chonnam National University / Faculty of Marine Technology

Temperature changes are one of the most significant environmental threats impacting global ecosystems. Abalone, Haliotis discus hannai, is a marine gastropod, and an important fishery and food industrial resource in Asia. To investigate the potential ecological risk posed by temperature stress, we measured biological responses such as survival rate, fatting rate of abalones, and histological changes in the Pacific abalone Haliotis discus hannai. Additionally, biochemical and molecular responses were evaluated in H. discus hannai exposed to various temperature gradients. The survival rate was reduced in abalones exposed to more than 26°C temperature stress, indicating a higher fatting rate of abalones and histological changes in the foot. Furthermore, increased antioxidant enzyme activities were observed in abalones exposed to relative high temperatures. The activities of superoxide dismutase were induced in a time-dependent manner after high temperature stress. Generally, heat shock protein 90 also increased significantly in H. discus hannai exposed to temperature gradients for 12 h. These results provide valuable information regarding stress responses to increased temperatures, in H. discus hannai; adverse biological and molecular outcomes could be utilized as risk assessments and stress monitoring of marine ecosystems under increased water temperatures.

WE059

Assessment of the response of Physa acuta (Physidae) to emerging contaminants

A.S. Sobrino-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia Laboratorio Alejandro Villalobos

Emerging contaminants (EC’s) are a diverse group of compounds which are included Pharmaceutical and Personal Care Products (PPCP’s) as soaps, creams, shampoos, deodorants, toothpastes, mouthwashes, detergents, drugs and perfumes. Also belong in this group products such as disinfectants and cosmetics. The studies on the effects of PPCP’s are scarce, for this reason the aim of this work was to evaluate the toxic effects of 6 products Personal Care (3 toothpaste: Colgate, Crest and Freska and 3 mouthwashes: Astringosol, Colgate and Listerine), 3 detergent (aspirin and paracetamol) on juveniles of Physa acuta gastropod to determine their sensitivity to these products. Toxicity bioassays were carried out with juvenile Physa acuta. The organisms were exposed to five concentrations of each product plus a control group, to determine the LC50 (lethal concentration 50). Furthermore the degree of lipid peroxidation in the tissues of gastropods was evaluated by measuring the concentration of MDA (malondialdehyde) in sublethal tests was (the most toxic to least toxic): Drugs > Detergents > Mouthwashes > Toothpastes. The results indicate that it is probable that a risk exists by the presence of the components of these products in the wastewater and discharged into aquatic systems, which could cause adverse effects to the populations of Physa acuta. Investigating potential combined toxic effects of Roundup and genetically modified maize in the aquatic environment of South Africa

K. Minnaar, North-West University / Zoology; H. Bouwman, North-West University / Environmental Sciences and Development

The global population is constantly increasing, with seven billion mouths to feed, and an estimated nine billion in thirty years. This increase in population places an increased demand on crop production, with a higher demand for crops and less workable land areas. The successful production of crops therefore, depends on the correct applications of inputs that will sustain the raising demands for food. This is achieved by combining technological advances, genetically modified (GM) crops, with the current practices of using herbicides and pesticides. The chemical broad-leaf herbicide Roundup has been reported to have effects on the development, and survival of invertebrates. However, GM crops, such as Bt-maize, are promoted as safer for the environment since no chemical pesticides are needed. This study aimed to determine the chronic effects of combining Bt-crop residues and Roundup on the development, fecundity, and survival of the freshwater gastropod snail Balanus tropicus. A static renewal toxicity test was used to expose one generation of B. tropicus to 50 cm³ of dried maize leaves (either non-Bt or Bt isolate) in 900 ml of synthesized freshwater, in different combination with 0.7 mg/L glyphosate. All treatments received the same amount of fish food every fourth day for the duration of the exposure, eliminating any nutritional differences between treatments. There was no indication of difference in the development, measured in growth, of the embryos irrespective of the presence of Roundup or Bt maize leaves. No decrease in survival of the embryos was found, indicating no adverse effects by either Roundup nor Bt on the development and survival of the embryo of B. tropicus.

WE062

Physiological and biochemical responses of Theodoxus fluviatilis to in situ exposure to antifouling paints

M. During, Applied Environmental Science; B. Eklund, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; A. Eriksson-Wiklund, Stockholm University / Applied Environmental Science

We investigated the effects of antifouling paints on a common snail species in the Baltic Sea – Theodoxus fluviatilis. The exposure was done in situ, in one marina and one guest harbor in the Stockholm archipelago, Sweden. In addition, a clean reference area was chosen at Askö, which also served as sampling site for the snails. Plastic cages (30 replicates per site), each containing 5 snails of different sizes and young tips of bladderwrack (Fucus vesiculosus), were placed in water at a depth of 1 m for 8 weeks. During this time the cages were checked periodically for dead snails and the fouling was brushed off to ensure good water exchange. The number
of egg capsules laid after two weeks was counted and the weight and shell growth were determined at the end of the exposure. Water and sediment samples were taken from the three sites for chemical analysis (Cu, Zn, TBT, Duron and Irgarol). The results show that the highest toxicity was towards the snails exposed in the marina, where the mortality was highest (i.e. 10 times higher than at the reference site), the reproduction rate was lowest (average 0.24 egg capsules/cage compared to 2.2 at the reference site) and the growth rate negatively affected (i.e. the fact that the water in the marina had the highest concentration of copper from all sites significantly contributed to the observed effects. Future analysis will be performed to investigate the biochemical responses of the snails, by measuring metallothionein, catalase and Cu/Zn superoxide dismutase. Our results show that the in situ exposure method is a good tool for observing the long term effects of marinans on gastropods, which are highly relevant test organisms, as they are the second most abundant group of animals.

**WE063 Preliminary experiments for the assessment of Littonia littorea sensitivity towards xenobenogenic chemicals**

P. Sanchez Marion, University of Aveiro & CESAM / INRSETE; S. Galante-Oliveira, C. Barroso, University of Aveiro / Biology Department CESAM

Prosobranch gastropods are widely used as sentinel species in endocrine disruption pollutants monitoring, in particular due to their sensitivity towards the biocidal tributyltin, the causative agent of imposex/intersex phenomena in these organisms. Other endocrine disrupting compounds (EDCs), such as birth control pharmaceuticals (synthetic hormones such as ethinyl estradiol), plasticizers and additives (such as PCBs, phthalates, bisphenol A), surfactants (such as alkylphenols), or insecticides (such as dioxin or metoxychlor), are gaining attention due to their generalized use and their presence in the environment. Except for tributyltin, the above EDCs have been described as estrogenic, and some have described effects of superimposition or even imposex (by negative feedback interactions) upon exposure of prosobranch gastropods to some of these chemicals. The aim of this study is to explore the effects that a model xenobenogenic chemical may cause in the periwinkle _Littonia littorea_, for its possible use in the risk assessment of this type of chemicals. For that, forty individuals (20 males and 20 females) were injected with 4 μL g⁻¹ fresh weight (fw) of a solvent control (DMSO) or ethinyl estradiol (EE2) at concentrations of 1 and 10 ng g⁻¹ fw. Non-injected control animals were also included and all individuals were maintained for 96 days in the laboratory. The following parameters were recorded in males: penis shedding, penis growth, and dimensions of prostate gland and seminal vesicle. In females, the dimensions of palial oviduct and ovispermator were registered. In addition, a histological analysis of gonads was done to assess the effects of the tested compounds on gametogenesis and possible anomalies in gonad tissues. The main observed effect of EE2 was an increase in the gametogenesis activity in males. A paradoxical result was an increase in penis growth rate in injected animals (DMSO, 1 and 10 ng EE2 g⁻¹ fw) compared with non-injected ones, questioning the use of DMSO as a carrier in injection experiments.

**WE064 Investigating priority substances with Potamopyrgus antipodarum**

P.T. Kajunjaki, T. Kaarinen, University of Helsinki / Department of Environmental Sciences; A. Rantalaiaen; O. Penttinen, University of Helsinki / Faculty of Biological and Environmental Sciences

New Zealand mud snail (_Potamopyrgus antipodarum_) is an invasive species which originates from New Zealand. It has spread from New Zealand to Australia, Europe, North America and Asia. The unique feature of these species populations is that there are no males present. The mud snail can reproduce through parthenogenesis. The offspring is grown inside of the female and hatched when the offspring is ready to imposex (by negative feedback interactions) upon exposure of prosobranch gastropods to some of these chemicals. Therefore, this study aimed to develop and optimise biomarkers for the assessment of metallothionein gene expression. Age-related assessment of toxicity indicated EC50 values from 0.17 to 0.62 mg/L for zinc and 0.95 to 19 mg/L for copper, and age 0 neonates were selected and used for subsequent standardized tests. The EC50 for age 0 neonates was 0.30 - 0.44 mg/L for zinc and 1.09 - 3.05 mg/L for copper. On-going work will establish the SOPs for use of _P. hawaiensis_ in ecotoxicity tests and provide a new model organism for investigating the effects of toxicants in important marine ecosystems. _Acknowledgement:_ The authors thanks FAPESF (project 2014/08832-9) and CNPq (400362/2014-7) for funding and CAPES for MCA fellowship, Prof. Augusto Luchessi, Silvia Berlanga and Sylvia Stuchi.

**WE067 The effects of selective serotonin reuptake inhibitors (SSRIs), serotonin and dopamine on chromatophore expression in the crustacean Crangon crangon**

A.S. Sobrino-Figueras, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologica Laboratorio Alejandro Villalobos; C. Caceres-Martinez, Universidad Autonoma de Baja California Sur

The Japanese oyster is an introduced species that grows in coastal systems of the Mexican Pacific, due to this resource has presented problems of survival, in the present study an evaluation of effect of some of the most abundant metals in the coasts of Mexican Pacific that are: Cd, Cr and Pb and their mixtures, was performed, to know its toxicity in veliger and pediveliger larvae and its effects on larval attachment is critical stage in the life cycle of this organism. Microbioassay were performed with duration of 72 hours, where the effect of metals separately and in combinations (Cd + Cr, Cd + Pb, Cr + Pb and Cd + Cr + Pb in the ratio 1:1) were tested, 5 metal concentrations (2.5, 1.23, 0.30, 0.078 0.019 ppm) in quintupled were used. This study presents the number of animals and number of larvae that were fixed to a substrate was determined. The toxicity of metals was (CL₄₉ the most toxic to least toxic): Cr > Cd + Cr > Cr + Pb > Cd + Pb > Cd + Cr + Pb > Cd > Pb. The interaction of metal mixtures was potentiation in all cases, with magnification factors ranging from 1 to 3. The percentage of settlement of larvae exposed to metals and mixtures was between 2 to 18 times lower than the controls. Metal concentrations with deleterious effects are very close to those reported in sediments from some sites within the Bay of La Paz B.C.S., San Luciano B.C., and Altaia Bay Sin. therefore it is required bioavailability assessments of metals on these sites.

**WE066 Development of the marine amphipod Parhyale hawaiensis as a model organism for ecotoxicology**

M.C. Artal, University of Sao Paulo - USP / Toxicology and Toxicology analysis; A. dos Santos; M. Eldridge, University of New Haven; T.B. Henry, Heriot-Watt University / School of Life Sciences; G.d. Umbuzeiro, FACULTY OF TECHNOLOGY -UNICAMP / LEAL

Development of model species for conducting ecotoxicity tests that are relevant to tropical marine animal and estuarine systems is urgently needed. The marine amphipod _Parhyale hawaiensis_ is already a model organism for developmental biology and other studies; and the worldwide distribution and importance of this species in tropical waters such as the coast of Brazil indicate that it could be an excellent model for ecotoxicity tests. Our objective is to introduce _P. hawaiensis_ as a model organism for ecotoxicology, develop standard operating procedures for husbandry and ecotoxicity testing, provide some toxicology information for this species upon exposure to reference toxicants and develop procedures for gene expression analysis. _P. hawaiensis_ were collected at Poço beach Itanhaem state of São Paulo, Brazil in 2010. Cultures were maintained in an incubator with controlled conditions that included continuous aeration, temperature 24°C ± 2, photoperiod of 12 h and salinity of 30±2. To obtain juveniles and neonates, the adults were separated from the culture and placed in smaller containers and neonates collected each day to ensure that neonates are of the same age. Toxicant (Zn²⁺ and Cu²⁺) exposures were conducted for 96 h, in static conditions, without feed, and one organism was exposed in 25 mL of solution with 10 replicates for each concentration. Mortality was recorded and the effect concentration 50% (EC₅₀) was estimated by Trimmed Spearman-Karber method. At sub-lethal toxicant concentrations, neonates were collected, preserved at -20°C, and used for development of standard operating procedures (SOPs) for total RNA extraction, reverse transcription, and Q-PCR for assessment of metallothionein gene expression. Age-related assessment of toxicity indicated EC50 values from 0.17 to 0.62 mg/L for zinc and 0.95 to 19 mg/L for copper, and age 0 neonates were selected and used for subsequent standardized tests. The EC50 for age 0 neonates was 0.30 - 0.44 mg/L for zinc and 1.09 - 3.05 mg/L for copper. On-going work will establish the SOPs for use of _P. hawaiensis_ in ecotoxicity tests and provide a new model organism for investigating the effects of toxicants in important tropical ecosystems. _Acknowledgement:_ The authors thanks FAPESP (project 2014/08829-7) and CNPq (400362/2014-7) for funding and CAPES for MCA fellowship, Prof. Augusto Luchessi, Silvia Berlanga and Sylvia Stuchi.

**WE065 Evaluation of the toxic effect of 3 metals and mixtures in veliger and pediveliger larvae of the Japanese oyster Crassostrea gigas**

A.S. Sobrino-Figueras, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologica Laboratorio Alejandro Villalobos; C. Cáceres-Martínez, Universidad Autónoma de Baja California Sur

The Japanese oyster is an introduced species that grows in coastal systems of the Mexican Pacific, due to this resource has presented problems of survival, in the present study an evaluation of effect of some of the most abundant metals in the coasts of Mexican Pacific that are: Cd, Cr and Pb and their mixtures, was performed, to know its toxicity in veliger and pediveliger larvae and its effects on larval attachment is critical stage in the life cycle of this organism. Microbioassay were performed with duration of 72 hours, where the effect of metals separately and in combinations (Cd + Cr, Cd + Pb, Cr + Pb and Cd + Cr + Pb in the ratio 1:1) were tested, 5 metal concentrations (2.5, 1.23, 0.30, 0.078 0.019 ppm) in quintupled were used. This study presents the number of animals and number of larvae that were fixed to a substrate was determined. The toxicity of metals was (CL₄₉ the most toxic to least toxic): Cr > Cd + Cr > Cr + Pb > Cd + Pb > Cd + Cr + Pb > Cd > Pb. The interaction of metal mixtures was potentiation in all cases, with magnification factors ranging from 1 to 3. The percentage of settlement of larvae exposed to metals and mixtures was between 2 to 18 times lower than the controls. Metal concentrations with deleterious effects are very close to those reported in sediments from some sites within the Bay of La Paz B.C.S., San Luciano B.C., and Altaia Bay Sin. therefore it is required bioavailability assessments of metals on these sites.
were used in a choice chamber experiment where they were presented with both black and white substrates in a tank and their settlement choices were observed over 20 minutes. Results revealed specimens became significantly darker on the darker background and lighter on the light backgrounds as one might expect with their ability to avoid predation. Significant differences from the control group were noted in the chromatophore scores between concentration, background colour and time. Discriminant analysis of chromatophore grades was able to cluster into groups based on colour of the substrate and highlighted that specimens on black substrates under the highest concentrations (1000ng/L Fx) had a chromatophore distribution matching those on white backgrounds. In addition, those on light backgrounds exposed to 10ng/L Fx were darker. All specimens preferred darker backgrounds and no significant difference in choice was observed after 20 minutes. The repeatability and further development of these tests are discussed.

**WE068**

**Do ground beetles differ in their response to chlorpyrifos?**

F.M. Bakker, Mixto Consultants; S.B. Dehelean, Mixto Trial Management BV

Ground beetles (Coleoptera: Carabidae) species are abundant in agricultural habitats and their surroundings. Being soil-inhabitants, soil dwellers and plant roammers, exposure to plant protection products occurs and impacts carabid abundance. Field observations indicate that species may be affected differentially. To investigate how differences in sensitivity at the level of the individual relate to the observed differences at the population level we assessed the response to varying doses of an organophosphate insecticide for different species of ground beetles and twoColeoptera. No toxicity testing (CS=micronised-encapsulated and EC=emulsifiable concentrate). Nine ground beetle species were collected from meadows situated in an agricultural landscape in SW France. The species were kept in captivity at the test facility until used for a test. Two species, Carabus auratus and Poecilus cupreus, produce sufficient progeny to enable testing of the larvae. Individuals were weighed and the test item was administered using topical application (1 µL drop). After dosing beetles were kept individually and mortality was assessed 72h later. With these data the LD$_{50}$ and NOED for the test species were calculated. Although the spectrum of species tested showed little overlap between the formulations, it is clear that the CS formulation was less toxic. For both products toxicity endpoints (LD$_{50}$ and LOED) were positively correlated with body weight and given the large differences in body weight these varied importantly. However, when expressed as dose per unit bodyweight there was no correlation with weight and values were of the same order of magnitude. Only Poecilus cupreus larvae fell outside the range (less sensitive). The results indicate that any carabid beetle can be used as an indicator for the sensitivity of other species and that species sensitivity distributions can be estimated from species weight distributions. The important next question is how species differ in exposure and how this relates to behavioural traits.

**WE069**

**Sensitivity of seven ground beetle species (Coleoptera: Carabidae) exposed to the organophosphate: chlorpyrifos.**

S. Sforzini, Università del Piemonte Orientale; A. Oldani, Renewable Energy & Environmental R&D Center - Istituto eni Donegani; F. Vago, eni S.p.A. / Direzione Ricerca ed Innovazione Tecnologica, Centro Ricerche per le Energie non Convenzionali; r. bortoli; Corporate; P. Cesti, ENI S.p.A. / Direzione Ricerca ed Innovazione Tecnologica, Centro Ricerche per le Energie non Convenzionali; L. Zaninetti, Syndial / Environmental Remediation; A.G. Viarengo, Universita del Piemonte Orientale / Department of Sciences and Technological Innovation DiSIT

Riparian areas play an important role in river ecosystems. In flood condition, the river water penetrates into riparian zones, carrying fine materials but also contaminants potentially present in the water body. In this study, a battery of biomarkers was utilised in the earthworm Eisenia fetida to assess the quality of riparian soils contaminated with organic and inorganic pollutants (such as PAHs, PCBs, DDT, Hg, Cu, Zn, etc.). In addition to high-level endpoints such as survival and reproduction, the responses of more sensitive sublethal parameters were investigated (i.e.) biomarkers of stress identifying effects at different levels of functional complexity evaluated in different cells and tissues of worms and ii) biomarkers of exposure to specific classes of contaminants (such as heavy metals and pesticides). When earthworms were incubated for 28 d in the contaminated soils, no effect was found in the survival of the organisms and the number of juveniles showed no alteration with respect to controls. Only a minimal non significant decrease in the species population was found. The results indicate that any carabid beetle can be used as an indicator for the sensitivity of other species and that species sensitivity distributions can be estimated from species weight distributions. The important next question is how species differ in exposure and how this relates to behavioural traits.

**WE071**

**Assessment of the population dynamics of Folsomia candida (Collembola) and its life history characteristics in relation to temperature**

V. Roeben, RWTH Aachen University; Institute for Environmental Research BioV; S. B. Dehelean, RWTH Aachen University / Institute for Environmental Research BioV; M. Ross-Nickoll, RWTH Aachen / Institute for Environmental Research BioV; A. Schaeffer, RWTH Aachen University / Institute for Environmental Research; T.G. Preuss, Bayer CropScience / Environmental Modelling

A recent study we developed the individual-based model FOLCAS which simulates the vertical dispersal and possible pesticide exposition of Folsomia candida (Collembola) in an agricultural soil column. Through this work it became obvious that detailed data on specific life history parameters of collembolans is still scarce. This work presents two experimental sections: an investigation of the population dynamics of F. candida and the scrutiny of life history characteristics in the offspring. In the former, the population dynamics of F. candida 10 individuals aged 9-12 days were placed on activated charcoal plates and kept at 20°C. The population was monitored for 11 months. Photos were taken at least on a weekly basis for counting. The results showed that the population size rapidly increased until it reached a density of about 22 ind/cm$^2$ after 10 weeks. Subsequently, the population size slowly decreased. In this phase no vital egg deposition was observed. After nine months population density reached a low and shortly after, F. candida started vital egg deposition again. Another approach using different food levels indicated that excessive food availability does not necessarily lead to higher population densities but rather decreases those in comparison to low food densities. In a second experiment life history parameters of F. candida exposed to temperature were investigated. In order to assess the life expectancy of F. candida 15 eggs were transferred onto activated charcoal plates. The eggs were stored at 4°C, 15°C, 20C, 24°C and 30°C. Subsequently, the hatching success and the longevity of the original generation were observed. This generation was transferred to fresh plates as soon as their offspring approached their size in order to distinguish the different generations. Egg masses at 4°C, 15°C, 20°C, 24°C and 30°C were observed at 24°C accompanied by reduced life expectancy in comparison to cooler temperatures (15°C-20°C). In addition, results of cooling experiments investigating overwinter survival will be presented. The results of the study will present new data for the otherwise well investigated Collembola species F. candida. In the next step the results will be used to refine species related procedures in the vertical distribution model FOLCAS, such as density-dependent mortality and reproduction and the life expectancy.
pesticides in Folsomia candida
T.F. Simes, Polytechnic Institute of Leiria / ESTM; G.R.M. S.C. Novaia, Polytechnic Institute of Leiria / ESTM and GIRM; T. Natal-da-Luz; J. Renaud, Department of Life Sciences; J. Sousa, University of Coimbra / Department of Life Sciences/CEP; T. De Boer, Vrije Universiteit / Ecological Sciences; D. Roelofs, Vrije Universiteit / Inst of Ecological Science; N.M. van Stralen, VU University Amsterdam / Department of Ecological Science; M.F. Lemos, Instituto Politécnico de Leiria / Dept Biology
Ecotoxicogenomics is a powerful tool to spot early molecular events involved in toxicant responses which are responsible for the adverse effects observed at higher levels of biological organization. Clear advantages come from linking the information of the phenotypic consequences of stress with the molecular changes occurred or suppressed after it. Taken together, phenotypic and transcriptomic data will help not only to unravel the mode of action of the stressor but also which components might be affecting the survival, growth, and reproduction of a given species, which may impact the population and ultimately the community. It is accepted that toxicity can be detected at the sub-cellular level earlier than it is observed at higher levels of biological organization. Folsomia candida is a common and widespread arthropod that occurs in soils throughout the world and has been used as a “standard” test organism for more than 40 years for estimating the effects of pesticides and environmental pollutants on non-target soil arthropods. This species is very sensitive to organic compounds and is among the most sensitive representatives of its taxon. In the present study, F. candida was used as test organism and its survival and reproduction was assessed (ISO 11267:1999(E)) after exposure to the herbicide glyphosate (30.8 % Montano®) and the fungicide chlorothalonil (38.8% Bravo®500) in a Portuguese agricultural soil. Organisms were then exposed for 10 days to the reproduction EC50 of each pesticide (glyphosate: 4.95 mg a.i./kg; chlorothalonil: 127.31 mg a.i./kg) as well as to control conditions, with sample collection at days 2, 4, 7 and 10. RNA was extracted from each pool of organisms and RNA sequencing yielded different sets of differentially expressed genes after exposure to each pesticide. Gene Ontology enrichment analysis identified distinct biological processes and molecular functions to be affected. Moreover, the pattern of gene expression changed over time. This work constitutes the first attempt to understand the mechanisms of toxicity behind the effects of glyphosate and chlorothalonil in soil invertebrates which can be used to develop a more effective set of tools to assess the early effects of such pesticides in a real scenario of soil contamination.
WE073
Toxicity Effect of Strain Bacillus thuringiensis. berliner on Domestic Silkworm (Bombyx mori)
Y-Ba, Nanjing Institute of Environmental Sciences; F. Shen, C. Liu, Nanjing University; C. Dai, Nanjing Normal University; Z. Shan, Nanjing Institute of Environmental Sciences MEP
As an extensively applied and the most successful pathogen used for insect control, Bacillus thuringiensis (Bt) presently occupies ~2% of the total insecticidal market (Bravo et al., 2011). Nearly 200 kinds of Bt products derived from different Bt strains have been on the market in the U.S. by 1998 (Schnepp et al., 1998). Bombyx mori which belongs to Lipodiptera Bombycidae is a commercially important insect for production of silk and recombinant proteins, and is also a good model lepidopteran (Goldsmith et al., 2005; Nagaraju and Goldsmith, 2002). It’s sensitive to Bt pesticides because of the similar classification with Bt targeted insects and may be poisoned by mulberry leaves contaminated by Bt pesticides used in mulberry field and surrounding farmland directly or indirectly through water, air and other means (Zhu et al., 2008). This study aimed to evaluate the toxicity of strain Bacillus thuringiensis. berliner (Bt.22945 for short) on silkworm Bombyx mori in four aspects including acute toxicity, proliferation toxicity, chronic toxicity and infectious toxicity. Using food intake method, high concentration of Bt strain didn’t show any change in toxicity may occur. Experiments that study the toxicity caused by Bt pesticides for non-target organisms, for example, the presence of imidaclopid had a negative effect on the population of aquatic invertebrates. Although we know that neonicotinoids may be toxic to non-target organisms their joint toxicity in mixtures of chemicals is the subject of very few studies. Toxicity testing of many chemicals at a time would be highly relevant, since that is how chemicals are likely to exist in the environment. The toxic effects of co-exposure of neonicotinoids as part of the complex mixture pollutants existing in the surface waters is currently completely unknown subject. This is the subject that this research proposal is going to tackle. Using the model organisms Lumbriculus variegatus and Chironomus riparius, the toxicity of neonicotinoids and other relevant contaminants is going to be tested in aquatic laboratory exposures. Sublethal endpoints such as survival rate, biomass and reproduction will be measured over 28 days and compared among different treatments of animals exposed to single neonicotinoids and in the presence of other chemicals. Over the 28 days treatments, the toxicity will be assessed every week by setting 4 timepoints. The hypothesis is that the addition of other chemicals (modifying the single-chemical testing conditions) may cause a synergetic effect, increasing the toxicity of neonicotinoids, although also other scenarios of antagonistic or no change in toxicity may occur. Experiments that study the toxicity caused by more than one substance are very relevant and can help to unveil what occurs in the real world. The results obtained will be meaningful in addressing the environmentally relevant situation when it comes to effects of pollutants and will be relevant for Finnish and European environmental authorities.
Challenging plasticity: can acidified conditions alter susceptibility to emerging contaminants in marine bivalves at different life-stages?

M. Munari, University of Padova / Biology; V. Matozzo, University of Padova / Department of Biology; F. Gagne, Emerging Methods, Environment Canada / Emerging Methods; G. Chemello, V. Riedl, L. Finos, University of Padova; M.G. Marin, University of Padua / Department of Biology

Climate change may alter behavior or susceptibility to pollutants, however this condition may be differently displayed in adult and early-life stages. To verify this hypothesis, the combined effects of seawater CO2-driven acidification and dielocfenac (a non-steroidal anti-inflammatory drug, NSAI) were investigated in the Mediterranean mussel Mytilus galloprovincialis and the Manila clam Ruditapes philippinarum. Using a flow-through system, adult bivalves were acclimatized to three pH values (8.1, 7.7 and 7.4) in 48 L glass cylinders for 7 days. Thereafter, they were maintained for 14 days at the three pH values and simultaneously exposed to environmentally relevant concentrations of dielocfenac (0.05 and 0.5 μg/L). Haemocyte parameters (total haemocyte count, haemocyte volume and diameter, Neutral Red uptake, haemocyte proliferation and lysozyme activity) and oxidative stress parameters (superoxide dismutase, catalase and cyclooxygenase activities, lipid peroxidation and DNA strand-break formation) in gills and digestive gland were analyzed after 7, 14 and 21 days of exposure to the experimental conditions. Results showed that cellular and biochemical parameters measured were more influenced by pH than dielocfenac with opposite patterns of variations between mussels and clams. R. philippinarum haemocytes showed reduced pinocytotic activity, compared to mussel haemocytes, suggesting a potential decrease in immune surveilance. Despite cyclooxygenase of their optimum NSAI performance, no signs of effects due to production. For fish, unveiled in this study, whereas pH significantly decreased COX activity in clams. Under flow-through conditions, fertilized eggs of R. philippinarum were exposed for 96h to two levels of pH (8.1 and 7.7) in the absence or presence of dielocfenac (0.5 μg/L). Larval survival and growth showed to be significantly affected by both reduced pH and dielocfenac; dramatic effects on shell development and prolonged tissue outside the shell were observed in larvae kept at pH 7.7. The highest levels of catalase activity were detected in low pHDielocfenac treated larvae, indicating oxidative stress, even though no significant evidence of increased lipid peroxidation was found. Overall results highlighted that seawater acidification enhanced susceptibility of bivalve larvae but not of adults to environmental concentration of dielocfenac, supporting the hypothesis that planktonic stages may be a population bottleneck under changing environmental conditions.

Multistress in aquatic environments: the big picture (P)

WE079

Evaluation of the genetic and physiological effects of Decabromodiphenyl ether (BDE-209) and Declorane plus (DP) in Mytilus galloprovincialis by a novel in vivo exposure via the dietary pathway.

E. Barón; A. Dissanayake, University of Plymouth / School of Biological Sciences; J. Vila, IDAEA CSIC Barcelona; J. Readman, School of Biological Sciences; A.N. Jha, Plymouth University / Biological Sciences; E. Eljarat, IDAEA-CSIC / Department of Environmental Chemistry; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants (FR) for many years and their concentration levels have been reported in different environmental and biotic matrices. Commercially, they were available in three different mixtures: Penta-BDE, Octa-BDE and Deca-BDE. Penta- and Octa-BDE mixtures were banned in 2008 in the European Union and production of Deca-BDE was banned by the end of 2013. The current demand of alternative halogenated flame retardants increased with the purpose of replacing PBDEs in polymer formulations. Declorane plus (DP) is a halogenated norbornene which was proposed by the EU as a replacement of Deca-BDE. Both classical and alternative flame retardants BDE-209 and DP have shown bioaccumulation and biomagnification potential. In general, there is a gap in the knowledge of the toxicological effects that these compounds may have. In addition, while some studies evaluated the effects of PBDEs in mussels (mainly focused on BDE-47) to the best of our knowledge this is the first in vivo exposure to DP in bivalves. Mussels were exposed to BDE-209 and DP at three concentrations for six days. BaP was used as in vivo positive control while acetone was used as negative control (n=9 per treatment). Exposures were conducted via dietary pathway, validated previously with BaP. Hydrogen peroxide was used as an in vitro positive control. Following exposures, clearance rate (CR) was measured and the alkaline COMET assay was performed immediately thereafter. CR were significantly higher than the control in all cases (p<0.001), but no statistically significant differences were observed among the different treatments at any of the three time points evaluated. The effects of exposure to BDE-209 and DP showed significant genetic damage at different concentrations in both cases. Furthermore, differences between the two compounds were observed at the different concentration levels of exposure. DP showed, at lower levels of concentration, similar DNA damage than BDE-209, which could suggest that it has a higher genotoxic potential. Moreover, our results showed that CR of the mussels was not affected at these doses. Therefore, results indicate that CR could be used as the most likely to be affected by these contaminants at the genetic level but not at the physiological level. Since DP has been proposed as an alternative to BDE-209, further investigation regarding its toxicological properties and mechanism are needed.

WE080

Salinity, pH and Arsenic effects on the biochemical responses of the manila clam Venerupis philippinarum

WE081

Combined impact of nutrition and pollution: the fatty acid profile modulates the tolerance to cadmium and methylmercury in fish

A. Lebrun, Institut des Sciences de la Vie; I. Neefs, B. Lemaire, J. Rees, Université Catholique de Louvain / Institut des Sciences de la Vie; Y. Laronduelle, UCLouvain / Institut des Sciences de la Vie; C. Debier, Institut des Sciences de la Vie Université catholique de Louvain

Aquatic organisms experience exposure to an external stressors which move them away from their optimum NSAI performance in terms of growth and reproduction. For fish, sub-optimal nutrition and heavy metals exposure represent two frequent stressors. There is a good knowledge about responses of fish to these stressors when considered individually. The lipid quality of the diet (ω-3 vs ω-6 fatty acids (FA)) has considerable impacts on the lipid composition of the organism, the FA metabolism, the membrane fluidity and the susceptibility to oxidative stress. The addition of heavy metals by heavy metals damages at both cell and organism levels (oxidative stress, altered membrane fluidity and FA metabolism, reduced growth and survival). Though both dietary lipid quality and heavy metals influence similar biological processes (e.g. redox homeostasis, FA metabolism), the interactions between these two parameters are poorly known in aquatic organisms. In that context, we aimed to explore the functional role of lipids in fish sensitivity to heavy metals (cadmium and mercury). First, an in vitro approach was conducted to assess whether the cellular FA profile has an impact on metal toxicity and to explore mechanisms behind such interactions. Practically, seven cell batches, from the rainbow trout liver cell line RTL-W1, differing in their FA profiles were developed by enriching the growth medium with specific FA. These modified cells were then challenged with cadmium and methylmercury. Results showed that mercury increases at cellular level the concentration of ALA and eicosapentaenoic acid (EPA) showed a particularly high protection against cadmium (EC50 3 times higher than in non-enriched cells) and methylmercury (EC50 4.5 times higher than in non-enriched cells) but none protected the cells against inorganic mercury. The FA profile of the cells would likely have influenced the tolerance of metals through modulating the membrane fluidity, the metal uptake and sequestration, as well as the activity of the pro- and anti-oxidative balance and preventing apoptosis. Based on the in vitro results, the protective potential of ALA and EPA against heavy metals is being investigated in rainbow trout juveniles to validate our observations at a higher level of biological organization. Overall, this study highlights the importance of food quality (at least in terms of its FA composition) in fish response to chemical contamination and provides novel perspectives to interpret contrasting toxicity studies.
Effects of carbon nanotubes and interleukins as a sentinel of marine pollution, through the use of suitable biomarkers.

This study indicates that ChE activity in blue sharks has the potential to be used as a sentinel species for pollution monitoring surveys, using biochemical biomarkers.污染并导致死亡。然而，LPO含量随As浓度和pH的增加而增加。虽然有机物和细胞毒性被认为是导致生物污染的关键因素，但在这两年中，我们所收集的数据尚不充分，以支持环境对污染物浓度和污染物种类的敏感性研究。Together taken, these findings provide a more precise understanding of ICNTs effects to mobilize and transport different contaminants to animals and how this could impact marine invertebrate late life over time.

Comparison of different extraction methods for the determination of booster biocides in marine organisms through liquid chromatography-mass spectrometry.

Since restrictive regulation of the antifouling agent tributyltin, the study of alternatives for As(III) and As(V) bioaccumulation through the use of tin-free antifouling paints is increasing due to high level of contamination in the marine and coastal ecosystems. Among all aquatic organisms, bivalves are considered to be good candidates to provide relevant information on environmental changes. Among all aquatic organisms, bivalves are considered to be good candidates to provide relevant information on environmental changes. The aim of this study was to assess the potential of the species of the genus Thais, commonly found in tropical and temperate regions, to be used as a sentinel species for pollution monitoring surveys, using biochemical biomarkers.

Using sharks as a model organism to assess the impact of pollution in different marine ecosystems, being a good candidate to provide relevant information on environmental changes. The results obtained revealed that none of the conditions induced mortality. However, the LPO content increased with the increase of As exposure concentrations, both at control conditions (pH 7.8 and salinity 28) and when combined with low pH or salinities of 14 and 42. The catalase activity showed a similar pattern when combining As concentrations and pH. Nevertheless, the activity of this enzyme strongly decreased when organisms were exposed to salinity 42 and the combination of As and salinity 42. Overall, this species provided relevant information on oxidative stress response to cope with changes in salinity and pH that may occur under climate change situations, associated with the presence of environmentally relevant concentrations of As in sediments.

Using sharks to assess the impacts of ocean contamination: a multibiomarker approach

P. glauca is an Atlantic species of bivalve mollusk of the genus Venerupis, commonly found in tropical and temperate regions. This study aimed to assess the potential of this type of molluscs as sentinel species for pollution monitoring surveys, using biochemical biomarkers.

We used sharks as a model organism to assess the impact of pollution in different marine ecosystems, being a good candidate to provide relevant information on environmental changes. The results obtained revealed that none of the conditions induced mortality. However, the LPO content increased with the increase of As exposure concentrations, both at control conditions (pH 7.8 and salinity 28) and when combined with low pH or salinities of 14 and 42. The catalase activity showed a similar pattern when combining As concentrations and pH. Nevertheless, the activity of this enzyme strongly decreased when organisms were exposed to salinity 42 and the combination of As and salinity 42. Overall, this species provided relevant information on oxidative stress response to cope with changes in salinity and pH that may occur under climate change situations, associated with the presence of environmentally relevant concentrations of As in sediments.

Effects of silver, nanosilver and their mixtures with functionalized carbon nanotubes on early life of sea urchins: from strong cellular impairment to morphogenic changes. Taken together, these findings provide a more precise understanding of ICNTs effects to mobilize and transport different contaminants to animals and how this could impact marine invertebrate late life over time.

Comparison of different extraction methods for the determination of booster biocides in marine organisms through liquid chromatography-mass spectrometry.

We assessed the potential of the species of the genus Thais, commonly found in tropical and temperate regions, to be used as a sentinel species for pollution monitoring surveys, using biochemical biomarkers.
D. Romero, University of Murcia / Marine and Environmental Toxicology; C. González-Fernández, Spanish Institute of Oceanography / Marine ecosystems; J. Campillo, Instituto Español de Oceanografía; L. Vinas, Spanish Institute of Oceanography; J. Bellas, Centro Oceanográfico de Vigo / IEO (Instituto Español de Oceanografía); A. Franco, Instituto Español de Oceanografía; M. Albentosa, Instituto Español de Oceanografía / Centro Oceanográfico de Murcia

Xenobiotics produce important immunological disorders in marine organisms, as in the mussel, *Mytilus galloprovincialis*. The study of these alterations in laboratory studies is really useful in order to identify biomarker responses of organisms to pollutants. Sensitivity of phenoloxidase activity (PO) to pollution suggests that this enzyme might be a reliable biomarker in monitoring programs. On the other hand, food availability in the environment seems to condition the biological responses to pollutants at different organization levels. Therefore, environmental trophic conditions could affect the immune responses to pollution. The experiment was designed to determine the relative importance of these two environmental variables: food availability and the presence of toxicants, on PO activity. Three experimental trophic conditions were simulated by regulating daily food ration during 45 days: low food condition (N1), medium food condition (N2) and high food condition (N3), which promoted negative, maintenance and positive mussel energy balances, respectively. In all cases, mussels were exposed to two nominal fluoranthene (FLU) concentrations (3 and 60 μg L\(^{-1}\)) for 3 weeks. After that, mussel haemolymph was extracted and analyzed spectrophotometrically, quantifying the production of dopacrome from L-DOPA and using SDS as elicitor. Multifactorial ANOVA showed a significant effect of nutritive condition on PO activity being this activity higher during the reproductive cycle.

**WE087 Fluoranthene toxicity in *Mytilus galloprovincialis* at different stages of gametogenesis measured by biochemical biomarkers**

C. González-Fernández, Spanish Institute of Oceanography / Marine ecosystems; M. Albentosa, Instituto Español de Oceanografía / Centro Oceanográfico de Murcia; J. Campillo, Instituto Español de Oceanografía; L. Vinas, Spanish Institute of Oceanography; A. Franco, Instituto Español de Oceanografía; J. Bellas, Centro Oceanográfico de Vigo / IEO (Instituto Español de Oceanografía)

Mussels, such as *Mytilus galloprovincialis*, are extensively used as sentinel species of pollution in marine coastal monitoring programs. Due to the large seasonal and spatial variability of marine ecosystems, large scale monitoring programs have some difficulties in the assessment of the effect of pollution on organism’s biochemical responses. It has been widely reported that biomarker responses show seasonal patterns due to exogenous (food availability and temperature) and endogenous (gametogenesis) factors. Generally, gonadal development of *M. galloprovincialis* mussels takes place between late autumn and winter, until the beginning of the spring, when the gonads is completely ripe and spawning occurs. During late summer and early autumn, there is a reserve storage that will be used for the beginning of the spawning season. The aim of this study was to evaluate the effect of fluoranthene on mussel reproductive status on their response to one polycyclic aromatic hydrocarbon (PAH): fluoranthene (FLU), measured by several biochemical biomarkers of exposure (superoxide-dismutase –SOD-, catalase –CAT-, glutathione reductase –GR-, glutathione peroxidase –GPx-, glutathione-s-transferase –GST-) and damage (lipid peroxidation –LPO-). For that purpose, mussels of the same size and from the same sampling site were exposed at two nominal concentrations when the mussels were in resting stage (October) and when the gonads were completely full (March).

Exposure was carried out at two nominal FLU concentrations (4 and 80 μg L\(^{-1}\)) for 3 weeks. Biological characterization of mussels from a biochemical, histological and anatomical point of view was also performed. Two-factor ANOVA analyses showed a significant effect of mussel reproductive status on several biomarker responses (SOD, CAT and GPx), whereas FLU exposure only affected GPx response. Mussels showed higher values on biomarkers measured during gonadal maturation than during resting stage. Since, in large-scale marine pollution monitoring programs, mussels display a wide range of reproductive status, even at the same sampling time, mussel reproductive status should be considered in order to carry out an accurate interpretation of biomarker results.

**WE088 Effect of copper in the freshwater bivalve Corbicula fluminea: Biomarker responses under laboratory and field in a short exposure time**

E. Bonnai, University of Cadiz / Chemistry/Physics; L.M. Buruain, Sao Paulo State University / Environmental Engineering, Escola Politécnica, Universidade Estadual Paulista / UNESP; G. Araujo, Universidad de Aveiro / Biologia; D. Abessa, Unesp / Marine Biology and Coastal Management; A.M. Sarmento, University of Huelva / Department of Geodynamics and Palaeontology

Copper is one of the best conductors of heat and electricity; it has important industrial applications, especially in a growing electric commerce. Its demand is forcing the development of different copper-containing compounds. Copper also affects many physiological processes, including immune responses. In particular, copper increases superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) activities, increases the production of reactive oxygen species and affects the immune responses to pollution. The experiment was designed to determine the relative importance of these two environmental variables: food availability and the presence of toxicants, on PO activity. Three experimental trophic conditions were simulated by regulating daily food ration during 45 days: low food condition (N1), medium food condition (N2) and high food condition (N3), which promoted negative, maintenance and positive mussel energy balances, respectively. In all cases, mussels were exposed to two nominal fluoranthene (FLU) concentrations (3 and 60 μg L\(^{-1}\)) for 3 weeks. After that, mussel haemolymph was extracted and analyzed spectrophotometrically, quantifying the production of dopacrome from L-DOPA and using SDS as elicitor. Multifactorial ANOVA showed a significant effect of nutritive condition on PO activity being this activity higher during the reproductive cycle.

**WE089 Seasonal variability of antioxidant biomarkers in mussels *Mytilus galloprovincialis* from the Spanish N-NW coast**

J. Bellas, Centro Oceanográfico de Vigo / IEO (Instituto Español de Oceanografía); J.A. Campillo, Instituto Español de Oceanografía; C. González-Fernández, Spanish Institute of Oceanography; A. Franco, Instituto Español de Oceanografía; M. Albentosa, Instituto Español de Oceanografía / Centro Oceanográfico de Murcia

Marine organisms are highly seasonal animals in relation to their physiology which depends, among other factors, on their annual cycle of reproduction. In bivalves, reproductive cycle is regulated by two main environmental factors: temperature and food availability. Specifically, bivalves are undergoing high variable environmental conditions. Integrated pollution monitoring carried out by the IEO along the N-NW coast of Spain has evidenced that the variability of the environmental conditions produce spatial differences in mussel condition which seems to mask the biomarker responses to pollution. Thus, there is a need to study the natural variability of biological responses used as pollution biomarkers at different seasons and in different sites in order to adequately assess the environmental pollution and biological responses. This study aims to assess the natural variability of some biomarker responses on the mussel *Mytilus galloprovincialis* in 5 different sites from the Spanish Marine Pollution Monitoring Program which are differentiated in their natural ecology and their anthropogenic pressure. The potential influence of environmental and endogenous factors that can cause biomarker’s seasonal fluctuations was examined. Biomarkers analyzed in this study are considered among the most useful biological tools applied in pollution monitoring programs, including exposure indicators (superoxide dismutase –SOD–, catalase –CAT–, glutathione reductase –GR–, glutathione peroxidase –GPx–, glutathione-s-transferase –GST–) and a damage indicator (lipid peroxidation –LPO–). Mussel biological characterization from a histological and anatomical point of view was also performed. Results evidenced that biomarkers were clearly influenced by the annual cycle (all of them were affected by the season) but also significant differences between sites were found in some biomarkers (GR and GST). Thus, not only environmental but also endogenous factors must be considered in monitoring programs in the study of biomarker responses.
zzebrafish embryo exposures, some sublethal side effects (e.g., edemas) were observed though survival was found to be the most sensitive endpoint. Mortality for zebrafish embryos coincided with hatching, suggesting the chorion provided some level of protection from UVA. Effect concentrations for UVA irradiated tests in both species tested were greater than an order of magnitude lower than exposures in the dark. Residue analysis at study termination indicated that observed effects were inversely related to measured pyrene concentrations in test media and that photo-transformation of accumulated pyrene contributed to the toxicity observed. The application of passive dosing in test design to maintain targeted, constant exposure concentrations provides an improved basis for developing consistent hazard data for phototoxicity test endpoints and subsequent model development to address interactions of chemical exposures and UV light in risk assessments.

WE091 Effects of nutritional stress on the freshwater detritivore, Gammarus fossarum: looking at the molecular level
B. SOHM, J. Arce-func, C. Crenier, LIEC / Université de Lorraine / CNRS; M. Dangier, LIEC; C. Cossé-Lelevé, LIEC / Université Lorraine; M. Labbé, LIEC / CNRS UMR 7146 - Université de Lorraine; B. Xueerab, University of Le Havre; S. Devin, LIEC - Université de Lorraine / CNRS / LIEC / CNRS UMR; F. Guerold, Universitè de Lorraine / Laboratoire Interdisciplinaire des Environnements Continentaux; V. Felten, LIEC

Nutritional constraints such as deficiencies in essential elements (e.g. nitrogen, phosphorus (P) or) molecules (e.g. essential fatty acids) impact living organisms at different levels from their growth, reproduction, energy storage to their response to biotic and abiotic stressors (contaminants). In forested headwater streams, mainly oligotrophic ecosystems, organisms are often subjected to low quality resources (i.e., P deficiencies). In such ecosystems, the detritivorous Gammarus fossarum represents a useful biological model as it plays a major role in leaf litter breakdown and it has an importance for various species (an already known be to negatively impacted (survival and growth rate) by a lack of phosphorus in leaf litter. Yet, underlying mechanisms are still unclear and an investigation at the molecular level should help to decipher these effects. In this study, we submitted G. fossarum to different litters (2 litter species along a P gradient) and monitored their physiological and molecular responses (gene expression: mRNAs analyses by RT quantitative PCR; proteins: western Bloting techniques). Genes and proteins were selected according to their involvement in different metabolic pathway and sequence or antibody availability. We first showed that diet P deficiency is correlated with a significant drop in energetic compounds (glycogen, total lipids), directly impacting the locomotor activity of G. fossarum. Moreover, we found that G. fossarum can grow with high P levels and that G. fossarum also exhibited two upregulated mRNA. Interestingly, these genes encode proteins involved in ATP synthesis (ATP synthase sub-unit) and in nucleotides phosphate exchange to maintain equilibrium between the concentrations of different nucleoside triphosphates. Both proteins are implicated in high P content compounds production and their gene expressions were correlated with P content increase, suggesting that gammarids can use P to store energy (ATP for lipogenesis and glycogenesis). Identification of deregulated proteins by western blot is still ongoing. We argue that molecular tools will permit to go deeper in the knowledge of metabolism and regulation pathways of living organism in response to environmental stressors. In particular, it should permit to investigate in more details the physiological state of organisms and help disentangling the impacts of multiple stressors on organisms (e.g., nutrient and contaminant).

WE092 Non-lethal heat shock increases tolerance to metal exposure in brine shrimp
J.L. Pestana, CESAM & University of Aveiro / Department of Biology and CESAM; S.C. Novais, Polytechnic Institute of Leiria / ESTIM and GIRM; P. Noronhail, GNT Université / Laboratory of Aquaculture Aquaculture Artemia Reference Center Faculty of Bioscience Engineering Rozier Gent Belgium; M.B. Vandegehechte, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; P. Bossier, Ghent University / Laboratory of Aquaculture Artemia Reference Center Faculty of Bioscience Engineering; K.A. De Soete, Ghent University / Université Libre de Bruxelles for Environmental Toxicology and Aquatic Ecology GHÉToxLab unit

Ecotoxicologists have been putting great efforts in methods to assess the ecological effects of complex mixtures to evaluate the environmental risk of contaminants under ecologically relevant scenarios of exposure. However, exposure to different stressors is not always simultaneous and evaluating the effects of sequential exposure is challenging as it involves the different concentration-response relationships we should also consider the potential effects that initial exposure to one stressor might have on the tolerance of organisms to additional stressors, i.e., stressor induced co-tolerance. In recent years researchers have focused on non-lethal heat shock (NLHS) and how it is involved in cross-tolerance in different organisms. This is because heat shock proteins(Hsp’s), acting as molecular chaperones, are induced and thought to protect organisms against different environmental stressors such as contaminants or pathogens and may be involved in the mechanisms of co-tolerance. However, there is limited information on the effect of NLHS on the subsequent tolerance of organisms to chemical exposure. Using gnotobiotic cultures of the brine shrimp Artemia franciscana we investigated: a) if NLHS causes an increased tolerance to subsequent metal exposure (Cadmium and Zn); b) if this co-tolerance is related to levels of 70Da-Hsp’s production and c) since both stressors have been shown to have epigenetic effects in crustaceans, we further investigated the possible epigenetic alterations (DNA methylation, histone acetylation) in A. franciscana exposed to non-lethal heat shock and metals. Our results show that exposing A. franciscana to a single NLHS (37 °C, for 30 min followed by 6 h recovery) markedly increased Cd and Zn tolerance. Moreover, we observed that both NLHS and metal exposure increased in the production of Hsp70, with Artemia exposed to both stressors suggesting that gammarids can induce tolerance to subsequent metal exposure with some evidences for the possible protective role of Hsp’s against both stressors. This work highlights the challenges of evaluating ecological effects of sequential exposures and call for discussion on the relevance and ubiquity of stressor induced co-tolerance.

WE093 The immunotoxic effects of Deepwater Horizon oil on Gulf of Mexico fish species
R. Rakeshita

In support of the Deepwater Horizon (DWH) Natural Resource Damage Assessment, we conducted a series of laboratory tests to investigate the compounds effects of polycyclic aromatic hydrocarbons (PAHs) and of Gulf of Mexico. Juvenile red snapper, southern flounder, Atlantic croaker, and red drum were exposed to oil via water accommodated fractions or spiked sediment for 2-9 days. During the oil exposure, we performed a bacterial challenge by moving the animals to tanks of seawater inoculated with Vibrio anguillarum (10^6 cfu/ml) for one hour. Fish were then monitored over time for mortality and behavior, and a subset of live animals were sacrificed for analysis at various timespoints post-bacterial challenge. Depending on the species, we measured growth (length and weight), red blood cell counts, gene expression, and microbiome composition. Our data analysis is ongoing, but our preliminary results demonstrate a dose-dependent immunosuppressive trend—from the molecular to organismal levels—in animals exposed to DWH oil.

WE094 Assessment of the genotoxicity and mutagenicity of hydric resources related with petroleum refining activities
M. Hoshina, UNEP - Institute of Biology / Biology; M.A. Marin-Morales, UNIVERSIDÉE; LaboraDUAL PAULISTA - UNEP / Department of Biology

Technological advances have many benef- we observed that there are several studies in literature on the impact of the petroleum refining activities in the Gulf of Mexico. We focus on the genotoxicity and mutagenicity of water samples from the northern Gulf of Mexico. We collected water samples from the northern Gulf of Mexico during summer 2014 and autumn 2014. From the results obtained, we could observe that the waters of the rivers analyzed, as well the refinery effluents contained in the Atibaia River showed the highest levels of Hsp’s. However, and concerning the epigenetic markers, we could not observe any effects of NLHS nor metal exposure on DNA methylation or histone acetylation in the first 12h of exposure. In sum, we have shown that NLHS can induce tolerance to subsequent metal exposure with some evidences for the possible protective role of Hsp’s against both stressors. This work highlights the challenges of evaluating ecological effects of sequential exposures and call for discussion on the relevance and ubiquity of stressor induced co-tolerance.
Cu, Cd or Co+Cd. The condition of the fish was monitored for 10 days, including behavioral and physiological metrics and also water quality monitoring (ammonia concentrations, metal analysis etc.). At the end of the exposure period, whole body and tissue metal concentrations were determined (i.e. brain, gills, liver, gut and muscle). The results showed that the fish which were exposed to the Cu were more sensitive than the ones exposed to Cd. Moreover, the toxicity in the Cu+Cd exposure was higher than in the Cd exposure. The feeding of the fish to metal exposure depended on the thermal history. In the absence of fish, the physiologically optimal temperature range (26-28°C) was neither the most metal sensitive nor tolerant acclimation temperature. A 32°C heat shock significantly improved tolerance against single and mixture metal toxicity compared to the ones not shocked or shocked at 34°C. The results also showed that short shock intervals leads to better tolerance against toxicity of metal concentrations. The results of these experiments show that the thermal prehistory and exposure temperature play an important role in determining the tolerance of zebrafish towards metal exposure. 

Heat shock treatment increases the tolerance but both the applied temperature and duration of the heat shock are critical in determining the final impact on metal tolerance. The observed effects are only partially related to the effect of temperature on metal uptake and accumulation and indicate that temperature acceleration and heat shock treatment result in homeostatic adjustments and up-regulation of certain defense system that also have an impact on metal tolerance. Analysis of gene expression and protein profiles is underway to reveal the molecular physiology that may provide explanations for the effects observed at the organismal level.

**WE096**

Chemical contaminants and biomarker responses in caged and native mussels (*Mytilus trossulus*) in the Archipelago Sea (SW Finland, Baltic Sea)  

K.K. Lehonen, Finnish Environment Institute / Marine Research Centre; R. Turja, Finnish Environment Institute, SYKE / Marine Research Centre; B. Budzinski, University of Bordeaux / UMR EPOQ Equipe LPTC; M. Dévier, LPTC-EPOQ, University of Bordeaux / Oceanic and Continental Environments and mussels

Mussels (*Mytilus trossulus*) were transplanted in cages along a suspected pollution gradient in the inner Archipelago Sea (SW Finland), northern Baltic Sea. The mussels were retrieved in early July and late August, 2011 and 2012 from the start of the experiment for the analysis of metal concentrations of chemical contaminants (polycyclic aromatic hydrocarbons (PAH), organometals, organochlorines, brominated flame retardants and trace metals) and a suite of biological endpoints including biomarkers (acetylcholinesterase activity, oxidative defence enzymes and metallothionein) and growth related parameters. Additional seasonal samples were collected from a mussel population at an alternative reference site where the exposure level was considered to be low. Seasonal patterns of metal concentrations in numerous contaminants (e.g., PAH), apparently due to loss of tissue mass during the long winter period, and also higher levels of various biomarkers responses (e.g., glutathione S-transferase activity). Marked differences in the accumulation of contaminants and biological variables were recorded between the caging sites, confirming the alleged contamination gradient. Aggregation and multivariate analysis methods over the biological effects data were able to distinguish the different caging sites from each other with linkages to the measured contaminant profiles. Temporal changes in the accumulation of contaminants and biological parameters were also observed, some of them (e.g., growth) being related to seasonal changes in prevailing environmental factors such as temperature and food availability. The results underline the importance of understanding the effect of seasonal factors on the natural condition and growth dynamics of mussels when assessing tissue concentrations of chemical contaminants and biological effects. The study supports the use of caged organisms in environmental monitoring and assessment of marine pollution in the Baltic Sea.

**WE097**

Impact of metal stressors on the polychaete "Branchipolynoe seepensis" and the host mussel "Bathymodiolus azoricus" from Mid-Atlantic-Ridge hydrothermal vents  

C. Cardoso, Institute of Marine ResearchCIMA-University of Algarve / IMARDOF/CIMA; G. Tomen, Norwegian Institute for Water Research (NIVA); C. Blasius, Institute of Marine Sciences / Instituto de Ciencias Marinas de Andalucía; A. Colaco, University of Azores / IMARDOF; R. Serrão Santos, IMAR Institute of Marine Research / MARE Marine and Environmental Sciences Centre; M. Bebiano, University of Algarve

Ventral hydrothermal vents are extreme environments with a variety of specific conditions that rendered them as one of the most productive and biology-rich deep-sea ecosystems. The fluids emanated from the sea floor are nutrient rich with high metal concentration that supports food web based on chemotrophic primary production. The vent commensal polychaete *Branchipolynoe seepensis* is commonly found in the paleal cavity of the vent mussel *Bathymodiolus azoricus*, the dominant specie along the Mid-Atlantic-Ridge (MAR). These vent worms have the ability to accumulate high levels of metals in their body absorbed from the surface of mussel gills. Mussels *B. azoricus* were collected from two sites from Lucky Strike Hydrothermal vent (1700m), Montségur-MS and Eiffel Tower-ET and from Rainbow hydrothermal vent (2300m) and checked for the presence of *B. seepensis*. Furthermore, to compare the interaction between both species, mussels were collected from Menez Gwen hydrothermal vent (MG2, MG3 and MG4) (850m) where polychaetes don’t exist. Metal concentrations (Ag, Cd, Co, Cu, Fe, Mn, Ni and Zn) were determined by ICP-OES in total and subcellular fractions of mussel tissues (gills, digestive gland and mantle) and in polychaetes whole body. Biomarkers of metal exposure, metallothioneins (MTs), and oxidative stress, namely antioxidant defence enzymes (catalase (CAT) and glutathione-s-transferase (GST)) and lipid peroxidation (LPO) were also determined in both species. Results showed different metal accumulation and accumulation, and indicate that temperature and metal tolerance. The observed effects are only partially related to the effect of temperature on metal uptake and accumulation and indicate that temperature acceleration and heat shock treatment result in homeostatic adjustments and up-regulation of certain defense system that also have an impact on metal tolerance. Analysis of gene expression and protein profiles is underway to reveal the molecular physiology that may provide explanations for the effects observed at the organismal level.

**WE098**

Temperature influences gene expression and phanthernen effect in biotransformation system of *Crassostrea brasiliana*  

Díd. Lima, UFSC; F.L. Zacchi, J.J. Mattos, Universidade Federal de Santa Catarina / Bioquimica; C.L. Bastolla, IMAR Institute of Marine Research MARE Marine and Environmental (NIVA); H. Budzinski, University of Bordeaux / UMR EPOQ Equipe LPTC; M. Dévier, LPTC-EPOQ, University of Bordeaux / Oceanic and Continental Environments and mussels

Accumulation of trace elements in liver and muscle: similarities and differences between control groups of 18°C, 24°C and 32°C under controlled laboratory conditions. A control group, without phenanthrene, was kept at each temperature. The levels of phenanthrene in the water was monitored by fluorescence at 250 nm excitation/360 nm emission in the first 24h. Two-way ANOVA was carried out to evaluate the effects of temperature and time of exposure (p < 0.05 was considered significant). The half-life of phenanthrene in water was inversely related (4.9h in 18°C; 2.1h in 24°C and 1.7h in 32°C). This result may indicate an increase in oyster metabolism, in higher temperatures, which increases the filtration rate. It was also investigated the relative levels of gene transcripts in the gill from control and exposed oysters by qRT-PCR. After 24h of exposure, gill cytochrome P450 (CYP2-like) from the exposed group at 24°C was significantly higher than the control group. It was seen interaction between phenanthrene and temperature, since control groups of 24°C and 32°C were significantly lower when compared to the control group. Similar pattern was observed for the following proteins: GST omega-1, GST microsomal (GST omega-1), GST microsomal (GST m), lipid peroxidation (LPO). Results showed significant differences between control groups of 18°C and 24°C, after 24h exposure. Superoxide dismutase (SOD-like), in 96h also showed differences between control groups of 24°C and 32°C. GST microsomal-like, sulfotransferase (SULT 1C1-like), Glutathione-S-transferase (GST like) and catalase (CAT-like) did not show differences. These results show that temperature by itself affect some of the tested genes and the response to phenanthrene exposure.

**WE099**

Accumulation of trace elements in liver and muscle: similarities and differences in Platyichthys flusus, Solea solea and Dicentrarchus labrax from two estuaries in Portugal  

J. Raimundo, IPMA / DIVOA; P. Pereira, Department of Biology and CESAM; M. Caetano, IPMA; C. Vale, IPMA / Instituto Portugueses do Mar e da Atmosfera; J.Neto , University of Coimbra / Marine and Environmental Sciences Centre; J. Lino Costa, universidade de lisboa / Instituto de Marinhos; A. A. Fonseca, DIVOA / Estuaries are important ecosystems due to their services and biodiversity. The presence of human pressures in the margins and watershed, often affects the Ecological Status of the system. Metals and metalloids are of particular concern due to their persistence in tissues of aquatic organisms. Despite the mobility of fishes in estuaries, accumulated toxic elements in liver and muscle may reflect availability in estuaries as well as in biochemical processes. Sixty two juveniles of *Dicentrarchus labrax* (DIVOA) from two estuaries in Portugal were obtained to evaluate the effects of *Platyichthys flusus*, *Solea solea* and *Dicentrarchus labrax* were obtained in two Portuguese estuaries (Mondego and Mira) of similar typology. Individuals were captured along the estuaries in winter and summer of 2012, and essential (Zn and Cu) and non-essential elements (As, Cd and Pb) were determined in muscle and liver. Differences on trace element accumulation were investigated in specimens of *Dicentrarchus labrax* and *Platyichthys flusus* (SULT 1C1-like), 50°C, and 100°C, and 120°C, respectively. In general, the metal concentrations in *Platyichthys flusus* were higher than in *Solea solea*, in muscle and liver. Differences in metal concentrations were higher in juveniles exposed to higher temperatures. The results pointed to a lower accumulation of Zn and Cu in muscles of *Solea solea* than in liver, while higher contrast was found for Cu, Pb and Cd reflecting the capacity of liver to retain these elements. Additionally, no significant differences

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were found for Zn concentrations in specimens between Mondego and Mira, maybe due to the essentiality of this element. *Platychirus flesus* and *D. labrax* from Mondego exhibited higher concentrations of Cu (muscle) and As (muscle and liver) than from Mira. Those geographical differences were not observed in *S. solea*. A clearer geographical pattern was obtained for Cd, showing higher concentration in liver of *P. flesus*, *D. labrax* and *S. solea* from Mira than from Mondego. The lack of differences in muscle reinforces the role of liver on Cd detoxification.

**WE100** Measurements of cardiac activity and markers of oxidative stress in gills of freshwater bivalves *Anodonta cygnea* L. exposed to a short-term salinity change used as functional load

T. Kuznetsov, Saint-Petersburg Scientific Research Center for Ecological Safety. Russian Academy of Sciences / LabBioelectronic Methods for Geocological Monitoring; A. Sharov , Russian Academy of Sciences / Institute of Biology of Inland Waters; S. Khолодевик, Russian Academy of Sciences / SaintPetersburg Scientific Research Center for Ecological Safety; G. Chuiiko, D. Pesnia , Russian Academy of Sciences / Institute of Biology of Inland Waters

The purpose of this study was to assess the effect of the salinity change as a form of standardized functional load for evaluation of physiological state of freshwater organisms. In this frame two different approaches were used simultaneously: a non-invasive cardiac activity monitoring and measurements of biochemical markers of oxidative stress (OS) in gills of freshwater bivalves *Anodonta cygnea* Linn. Bivalves were exposed in laboratory to saline solution of sodium chloride at a concentration of 3 ppm for 60-120 min and then the salt water was replaced by fresh water. Cardiac compensatory response to such type of load and dynamic of heart rate (HR) after replacement of water to natural one were registered by on-line heart rate monitoring system simultaneously in 8 mollusks. Exposure caused increase in HR of bivalves from the beginning of the salinity change and during all the time of exposure. Replacement of salt water by ambient one caused recovery of HRs in 1-2 h according to bivalve’s physiological state. Exposure of bivalves to saline solution did not cause any changes in the content of malonic dialdehide (MDA), a lipid peroxidation (LPO) products, reduced glutathione (GSH) and activities of catalase (CAT), glutathione reductase (GR) and glutathione-S-transferase (GST). However, when saline solution after exposure of 60 and 120 min was replaced by fresh water, significant increase of MDA was revealed after 15-min recovery period. Simultaneously induction of activities of CAT and GST were reported. After 45-60 min of recovery these parameters returned to normal levels and were not changed till the end of the experiment. At the same time, GSH content and GR activity was significantly increased after 120 min in bivalves after recovery to ambient freshwater. These results indicate that a short-term increase in water salinity of 2-3% doesn’t induce OS in bivalves. However, returning bivalves to fresh water induces LPO and activates the antioxidant defense system in their gills. As a whole, changes of markers of OS were not great and quickly return to normal after an hour of salinity recovery. This study proves that a short-term osmotic stress (as a result of change in the salinity of medium) don’t pose threat for this species of bivalves and could be used as a functional load to evaluate physiological state of the organism of hydrobiots.

**WE101** The Dreissena polymorpha invasion in Europe: the scale of the problem and a novel control strategy.

S. Meehan, TSGE Consulting; A. Shannon, TSGE; F. Lucy, The Institute of Technology Tralee

Highly invasive zebra mussels (*Dreissena polymorpha*) in European freshwaters have rapidly spread to lakes, rivers and canals. Colonisation has been on natural substrates, freshwater biota and man-made infrastructure. The availability of an effective, yet targeted, mussel control product to replace those currently used (such as chlorine and other non-specific biocides) is urgently needed to mitigate mussel fouling impacts in drinking water plants and other facilities which are immersed or use fresh water resources. The efficacy of a naturally occurring soil bacteria, *Pseudomonas fluorescens* CL 145A, in controlling *D. polymorpha* was first demonstrated by Dr. Dan Molloy, formerly of the New York State Museum. Initial tests by Molloy showed that *Pseudomonas fluorescens* CL 145A is highly specific to *D. polymorpha* mussel. Non-mussel testing was carried out to further evaluate the specificity of CL 145A cells with respect to other common biota in European freshwater systems. This included protected and known vulnerable species in order to establish the potential for any impacts on native aquatic vertebrates and invertebrates. Two field trials were conducted to demonstrate the efficacy of CL 145A in industrial facilities (using onsite ‘bioboxes’ at a water treatment plant) and in natural freshwater systems (in infested open water (a canal)). Effects on water quality during and after treatment were also monitored. Both trials achieved high levels of zebra mussel control (up to 81% adult mortality) and provided insights into practical applications. Methodology approach using effect-based monitoring tools to link surface water quality, ecotoxicity and pollutants availability

N. Roig, Universitat Rovira i Virgili; J. Sierra, Universitat Rovira i Virgili / Soil Science Unit; I. Moreno Garrido, Instituto de Ciencias Marinas de Andalucia (CISC); E. NIETO, INSTITUTE FOR MARINE SCIENCE OF ANDALUSIA ICMANCSIC / ECOLOGY AND COASTAL MANAGEMENT; M. Hampel, Instituto de Ciencias Marinas de Andalucía / Consejo Superior de Investigaciones Científicas; M. Nadal, Universitat Rovira i Virgili / Laboratory of Toxicological Environment and Health; J. Blasco, Inst. Ciencias Marinas de Andalucía / Instituto de Ciencias Marinas de Andalucía; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering

According to the European Water Framework Directive, the assessment of the ecological status of freshwater systems is based on biological and hydromorphological quality. Some studies have observed that biological status is not always in coherence with chemical status, maybe due to the adaptation mechanisms of aquatic organisms under chronic chemical exposure. In these situations, ecotoxicity tests could be useful to resolve these divergences. The use of effect-based monitoring tools has been mentioned in the context on the Common Implementation Strategy (CIS) focused on regulated substances, which involves that many substances are not considered and their contributions to toxicity are lacking. The general aim of this study is to design a methodology to categorize the ecotoxicological status of rivers. The specific aims are: to compare the effectiveness of different ecotoxicity tests performed with freshwater sediments; to evaluate the relation between ecological status, pollutant, recent sources, their availability, and water and sediment samples. T. Kuhn, TUEB develop a general methodology to evaluate the relative contribution of the main pollutants to the toxicity in order to improve management tools. Two sampling campaigns were carried out in 2013 and 2014 within the Ebro River (Spain) watershed. Thirteen composite samples of sediments were collected and data on the chemical and ecological status has been achieved. The ecotoxicity of pore water and whole sediment was evaluated using microorganisms, *Daphnia magna*, *Pseudokirshneriella subcapitata*, *Daphnia magna* and *Chironomus riparius*. In addition, the trace metals bioavailability was calculated by a sequential extraction according to the BCR method. To distinguish the potentially toxic fraction associated to trace metals burden of sediments, an analysis of acid-volatile sulphide and simultaneously extracted metals was performed. The results indicate that the ecotoxicological approach in most of the analyzed sediments is in agreement with their ecological status. This study demonstrates that the integration of chemical, biological and ecotoxicological analyses could be crucial to understand the hazard of pollutants in aquatic ecosystems, especially, in freshwater sediments. Moreover, it corroborates that cost-effective and rapid screening short-term bioassays could be useful to determine the surface water ecotoxicological status.

**WE103** Spatial hotspots for the introduction of crop plant residues into surface water bodies - a procedure for stream type prioritization

R. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Umweltbewachungsamt; D. Elsaesser, University of Koblenz-Landau / Umweltbewachungsamt; M. Schuhmacher, Rovira i Virgili University / Toxicology and Environmental Health; J. Blasco, Inst. Ciencias Marinas de Andalucía / Instituto de Ciencias Marinas de Andalucía; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering

Spatial hotspots for the introduction of crop plant residues into surface water bodies (or at least into small surface water bodies, at least in stream type D) were found for Z. mays and *P. flesus* in the Mondego stream. Due to the essentiality of this element. The Dreissena polymorpha invasion in Europe: the scale of the problem and a novel control strategy. Highly invasive zebra mussels (*Dreissena polymorpha*) in European freshwaters have rapidly spread to lakes, rivers and canals. Colonisation has been on natural substrates, freshwater biota and man-made infrastructure. The availability of an effective, yet targeted, mussel control product to replace those currently used (such as chlorine and other non-specific biocides) is urgently needed to mitigate mussel fouling impacts in drinking water plants and other facilities which are immersed or use fresh water resources. The efficacy of a naturally occurring soil bacteria, *Pseudomonas fluorescens* CL 145A, in controlling *D. polymorpha* was first demonstrated by Dr. Dan Molloy, formerly of the New York State Museum. Initial tests by Molloy showed that *Pseudomonas fluorescens* CL 145A is highly specific to *D. polymorpha* mussel. Non-mussel testing was carried out to further evaluate the specificity of CL 145A cells with respect to other common biota in European freshwater systems. This included protected and known vulnerable species in order to establish the potential for any impacts on native aquatic vertebrates and invertebrates. Two field trials were conducted to demonstrate the efficacy of CL 145A in industrial facilities (using onsite ‘bioboxes’ at a water treatment plant) and in natural freshwater systems (in infested open water (a canal)). Effects on water quality during and after treatment were also monitored. Both trials achieved high levels of zebra mussel control (up to 81% adult mortality) and provided insights into practical application and possible control techniques. No long term effects on water quality were observed. This research demonstrates the potential to replace traditional control methods (for example as off label use of chemicals such as chlorine) with targeted novel biological biocides, thus helping to reduce the impact of *D. polymorpha* in the environment while protecting vulnerable native aquatic species. Keywords: Zebra mussel, invasive species control, biological biocide
to a prioritization of potentially most exposed and sensitive stream types. The suggested procedure should support the authorities by identifying potentially exposed and sensitive habitats and species living in these habitats that may be particularly vulnerable to GM exposure.

**WE104**

Photo-Induced Toxicity of Deepwater Horizon Oil to Native Gulf of Mexico Fish and Invertebrate Species

J.M. Morris, Stratus Consulting; A.P. Roberts, University of North Texas; J. Oris, Miami University / Department of Biology; C. Lay, Stratus Consulting; J. Lipton, Stratus Consulting, Inc.

In support of the Deepwater Horizon (DWH) Natural Resource Damage Assessment, we performed over 50 bioassays and related chemical characterizations to determine the photo-induced toxicity of DWH oil to native species in the Gulf of Mexico. Several tests combining waterborne or sediment exposures of early life stage organisms to oil followed by exposure to natural sunlight were conducted on 10 different species of fish and invertebrates. These tests included a range of life stages spanning from gamete exposures to exposures of gravid females. Exposure to UV light following oil exposure generally increased the toxicity of the oil by at least 10 fold during very short duration experiments (1-24 h). Analyses of these data are ongoing but we will discuss effects levels for a range of species including oysters, fiddler crabs, blue crabs, mahi-mahi, red drum, speckled sea trout and mimnows. Additionally, we will discuss exposure chemistry, testing methods, and our meta-analysis of this dataset.

**WE105**

Survey and Analysis of Data Available About the Water Quality of Micro Basin Lagoons in IlhaSolteira - SP, Brazil

J.N. Oliveira, Unesp / Civil Engineering; K.T. Zambrano, Unesp / Civil Engineering Dept

The lack of urban growth, without proper planning about the use and occupation of land, generate environmental impacts related to erosion, siltation and overload of water resources located in urban and peri-urban area of the city, compromising the water quality. Although, at world level, Brazil is a privileged country due to its large availability of water resources, many regions have water shortage problems that highlight the importance of sustainable management and preservation, especially in urban streams – victims of the growing population and misuse of resources – that suffers with the increasing flow and removal of riparian vegetation. In view of this, the survey and analysis of data about water quality of the micro basinLagoas, located in the municipality of IlhaSolteira, north-west of São Paulo, allow studies concerning the magnitude of potential impacts in the last decade in order to undertake steps to stop this trend from propagating. A survey of published studies researches about studies on water quality was performed on the bank of dissertations from the Faculty of Engineering of IlhaSolteira. The search made used as keyword ‘Stream’, without a base year definition and resulted in 27 studies. Then, based on the studies reading, a new selection led to the identification of three dissertations about the subject. The authors considered a total of 8 study points in the micro basin, and analyses cover the period from 2002 to 2013. In general, in 2002 the closest points to urban areas showed elevated concentrations of turbidity, apparent color, solids, colloforms and BOD, as well as low levels of dissolved oxygen (DO), and most of the results disagree with the CONAMA Resolution. In 2011 was observed a significant increase in turbidity and solids parameters, as well as reduced levels of DO. In 2013, the parameters are still in disagreement with the Resolution and evidence the resource contamination and increase of erosion processes. This analysis emphasizes the need of measures such as the implementation of retention watersheds, improvements in use and conservation of the soil through techniques as management of level curves, clandestine sewage elimination, recovery of riparian forests and environmental education of society, in order to avoid permanent impairment of the watersheds water resources, that make impracticable the use and bring risks for health of the riparian population.

**WE106**

Preliminary assessment of the water streams through the electrical conductivity in IlhaSolteira - Brazil

J.L. DeFreitas, Unesp / Civil Engineering; C.M. Brito, Unesp / Engineering Civil

Since 2009 the sub-basin of stream Sem Nono, with an area of 3.47 km², was already completely urbanized. The stream has ephemeral characteristics and its base flow is greatly reduced during dry seasons. The flow also varies during the day, increases a little after 10 am possibly due the addition of sidewalks cleaning water. The objective was to evaluate preliminarily the electrical conductivity and pH of the water during the collected 30 surface water samples in March. Initially with an interval of 5 minutes and then 15 minutes. The analyzes were performed in the laboratory with bench pH meter Hanna pH 21. The pH ranged from 8.03 to 8.79 remained within the acceptable range according to the Resolution No. 357 of CONAMA for streams class 2. In comparison, the electrical conductivity remained within the standards suggested by the state agency CETESB only until 10 hours, increasing 68.8 μS/cm to 863.6 μS/cm. From that time there was great growth, jumping from 107.6 μS/cm to 2135.5 μS/cm. Relatively high values were also found in the sub-basins of the streams of Leucena and of Cinturão Verde, despite being adjacent, have different occupation characteristics, with less urbanized area. The average values were respectively 163.2 μS/cm and 222.5 μS/cm in August 2009. Thus it is evident that the area is impacted there is no way to know more about the source as it is not possible to infer whether the urbanization contributes or not to the high values that we found. However, apart from the impact suggested by conductivity values, field observations were made and allowed us to see that the water is usually anoxic due to low flow basis and possibly because illegal sewage connections.

**WE107**

Risk survey of a chemical contamination chronicles in the space of the Marina of LA ROCHELLE: understanding of the adaptive processes of marine species

M. Breitenester, LIENs UMR7266 2 rue Olympe de Gouges 17000 La Rochelle; D. Flechet, LIENs UMR 2 rue Olympe de Gouges 17000 La Rochelle; A. Fontanaud, Port de Plaisance de La Rochelle 17000 LA Rochelle; C. Churlaud, LIENs UMR7266 rue Olympe de Gouges 17000 La Rochelle; h. thomas,

The evaluation of the impact of pollutants in the environment is at present one of major concerns and it with the aim of reaching to the good chemical and ecological state of the littoral circles. In this context, the objective of this work will try to better understand the natural and anthropological phenomena which affect the functioning of a Marina of La Rochelle and its close environment: pollutants (hydrocarbons, heavy metals) which impact on the specific fauna but also on the neighboring zones and on the zones of relargage of the muds of dredging. Indeed, the harbour spaces constitute zones of interesting studies in terms of obvious environmental requirements on restricted surfaces. So, the biological effects of chemical contamination in the nearest area of a Marina in La Rochelle accompanied with trying to develop an approach multi-biomarkers on specific marine species of this site and consider the environmental variability (climatic & anthropic). In this context, animals were collected on diverse intra-harbour stations in order to (1) quantify the content of the inorganic contaminants in the marine bodies, and (2) realize the follow-up of indicators of feature to identify an indicator species such as bivalves (oysters & clams) of intra-harbour pollution. These studies uses the platforms and technical site of LIENs will and consider biochemical, physiological and/or genetic descriptors to animals. So, parameters of detoxification and enzymes of the oxidative stress were used as “alarm system” susceptible to detect a disturbance before the appearance of irreversible pathological signs. The obtained data was valued in association with the local actors (region councils) and in connection with the communications office of the Marina of La Rochelle.

**WE108**

Changes of sessile organisms around the Fukushima Dai-ichi Nuclear Power Plant after nuclear accidents in March 2011

T. Kashino, National Institute for Environmental Risk Research; K. Kodama, National Institute for Env. Studies / Center for Environmental Risk Research; T. Akatsuka, National Institute for Environ Studies; H. Shiraishi, National Institute for Environmental Studies / Research Center for Environmental Risk

To investigate possible adverse ecological effects caused by accidents of Fukushima Daishin Nuclear Power Plac (1F) after the earthquakes and Tsunami in March 2011, we have conducted field surveys at intertidal zones since December 2011. We carried out field surveys at intertidal zones of 43 sites along the coastal line of eastern Japan from August to March 2012 and those on sessile organisms at 7 sites by 50cm x 50cm quadrate method in May/June 2013 and June 2014. The number of species of intertidal biota seemed to get smaller as the site got closer to 1F in 2012. No rock shell (Thais clavigera) specimens were collected at 8 sites, located around 1F of Fukushima prefecture, in 2012. Because the rock shell specimens were collected at many sites in Miyagi and Iwate prefectures in 2012, where enormous Tsunami attacked, it is unlikely that small number of intertidal species and no rock shell specimens around 1F were caused only by Tsunami. Results on surveys on sessile organisms by quadrate method in 2013 showed the number of species, the number of individuals per square meters and whole wet weight of organisms per square meters were much lower at sites in the south of 1F than those in other sites, especially for Arthropoda. The densities of sessile organisms per square meters at sites in the south of 1F in 2013 were much lower than those in 1995. Cooling water, contaminated by various radionuclides and harmful chemicals, leached from the nuclear reactors directly to the sea in March-April 2011 may have given any impacts to intertidal biota including rock shell populations and sessile organisms around 1F. Results on surveys on sessile organisms by quadrate method in 2014 will be also shown to discuss about their temporal changes.

**WE109**

Impact of methylmercury exposure on marine fish ecophysiology under ocean warming and acidification

S.d. Francisco, MARE Marine and Environmental Sciences Centre; M.S. Pimentel, J.R. Paula, University of Aveiro; V. Madeira, T. Repolho, Faculdade de Ciências da Universidade de Lisboa / MARE Marine and Environmental Sciences Centre; A.M. Marques, Portuguese Institute for the Sea and Atmosphere / Division of Aquaculture and Upgrading; A. Maulvaui, IPMA, L.P. / Division of Aquaculture and Seafish Upgrading; R. Rosa, Faculdade de Ciências da Universidade de Lisboa / MARE Marine and
Environmental Sciences Centre
Climate change predictions indicate that oceans will become up to 4°C warmer and ocean pH will drop 0.4-0.5 units. These changes are expected to drive deleterious impacts on marine organisms’ distribution and abundance. Moreover, ocean warming and acidification are both known to exacerbate the toxicity of pollutants (e.g. mercury) to many life history stages. There are considerable evidences that mercury levels (Hg) in the environment have greatly increased in the past century. Although the mechanism by which the environment is contaminated is imbalanced, some is contributed to the highly toxic methyl mercury (MeHg), which bioaccumulates in fish. Elevated MeHg concentrations is known to increase neurodevelopmental abnormalities which may trigger deleterious effects on fish behavior, performance and health. The combined impacts of ocean warming, acidification and mercury pollution on marine species are not known. In this study, we investigated, for the first time, a set of biological responses to the combined effect of methylmercury exposure, warming (+4°C) and acidification (∆pH = 0.5 units) in meagre (Argyrosomus regius) juveniles, namely morphological, physiological and biochemical changes during 30 days. The potential cascading effects on the marine food webs are also discussed.

WE110 Do warmer winter temperatures make damselfly larvae more sensitive to pesticide exposure? H. Arambourou, Irstea Lyon / Laboratoire décontoxicologie; R. Stoks, University of Leuven / Department of Biology
Global warming is expected to increase winter temperatures resulting in shortened duration of ice. Invertebrates respond at northern latitudes. Given that higher water temperatures during winter are generally associated with increased metabolic rates, this could result in a stronger depletion of energy reserves. Therefore, under global warming freshwater invertebrates may become more sensitive after winter when exposed to pesticide sprays in spring. In the present study, we tested this hypothesis in the common damselfly Ischnura elegans using a common-garden exposure. In order to test whether the combined effects of winter temperature rise and subsequent exposure to the widely used pesticide chlorpyrifos. To assess the potential mediatory role of local thermal adaptation, we applied these treatments to larvae of replicated low- and high-latitude populations in Europe. In the larvae we measured two life-history traits (mortality and growth rate) and three biochemical markers (lipid content, acetylcholinesterase activity and phenoloxidase activity). Winter mortality was lower and growth rate higher during the simulated warmer winters (8°C) than during the simulated cold winter (4°C) at the high latitude. In line with a pattern of thermal adaptation, low-latitude larvae suffered a higher mortality during winter than high-latitude larvae. Subsequent chlorpyrifos exposure at 20°C induced a significant increase of mortality and decrease of growth rate, and this irrespective of the previously experienced winter temperature and the latitude of origin.

WE111 Combined dietary effect of P and Zn on reproduction of D.magna C. Gap, Department of Earth and Environmental Sciences; K. A. De Schamphelaere, University of Ghent / Laboratory for Environmental Toxicology and Aquatic Ecology GEEnToxLab unit; E.E. Smolders, Katholieke Universiteit Leuven / Division Soil and Water Management
Phosphorus (P) and lipids (including sterols and essential 3- and 6- polyunsaturated fatty acids (PUFAs) provide a substantial contribution to the food quality for invertebrates and are vital for somatic growth, survival, and reproductive success. Recent study evidence the combined effects of winter temperature rise and subsequent exposure to the widely used pesticide chlorpyrifos. To assess the potential mediatory role of local thermal adaptation, we applied these treatments to larvae of replicated low- and high-latitude populations in Europe. In the larvae we measured two life-history traits (mortality and growth rate) and three biochemical markers (lipid content, acetylcholinesterase activity and phenoloxidase activity). Winter mortality was lower and growth rate higher during the simulated warmer winters (8°C) than during the simulated cold winter (4°C) at the high latitude. In line with a pattern of thermal adaptation, low-latitude larvae suffered a higher mortality during winter than high-latitude larvae. Subsequent chlorpyrifos exposure at 20°C induced a significant increase of mortality and decrease of growth rate, and this irrespective of the previously experienced winter temperature and the latitude of origin.

WE112 Effect of pathogen infection on Caenorhabditis elegans stress response to various environmental contaminants Q.N. Choudhry, N. Chatterjee, University of Seoul; J. Choi, School of Environmental Engineering Graduate School of Energy and Environmental System Engineering
In most of toxicology studies, responses of organism are investigated to a single chemical exposure. However, in a real environmental condition, most of organisms are simultaneously faced to various chemical and biological stresses. We have been focusing how the combined stress situations could induce the stress response in an organism. To explore this phenomenon we carried out a study designed using Caenorhabditis elegans, which is a common model in toxicology and immunology. Worms were infected with pathogenic bacterial Pseudomonas aeruginosa and were subsequently challenged to chemicals for 24 hours and mortality was measured. To determine how these treatments would synergize or antagonize, we tested a range of categories of chemicals, including organic compounds (nonylphenol, benzene, DEHP, PFOA, PFOS, BDE-209), heavy metals (Cd²⁺, Pb⁺⁺, Cu⁺⁺) and nanomaterials (silver nanoparticle, silica nanoparticle, titanium dioxide nanoparticle, carbon nanotubes, graphenes) were screened. Among the tested chemicals AgNP showed the rescue effect whereas cadmium, lead and nonylphenol treated worms were found more sensitive with pre-infection. Other chemical mixtures such as benzene, copper, cadmium + PFOS, AgNP did not show any interaction. Subsequent chlorpyrifos exposure at 20°C induced a significant increase of mortality and decrease of growth rate, and this irrespective of the previously experienced winter temperature and the latitude of origin.
front ingestion/filtration of $C.~rachovskii$ in concentrations 10μg/L and 20μg/L were observed significant reduction ($p < 0.05$) the concentration of cyanobacteria, while the concentration 40μg/L did not was observed significant differences. Results of morphological analysis revealed the existence of gill microsphere, both in $Oreochromis$ sp. as for $G.~brasiliensis$ validating the possibility of $G.~brasiliensis$ can be used as natural control of cyanobacteria. These results are important for knowledge management and restoration of eutrophic aquatic ecosystems based on species interactions.

WEI18
Can elevated pesticide concentrations in sediments indicate past acute pollution events

V. J. Pettigrove, The University of Melbourne / Zoology; S. Mohr, Umweltbundesamt / IV; L. Reiber, Helmholtz Center for Environmental Research; S. Marshall; B. Gagliardi, The University of Melbourne / Zoology; S. Meinecke, Umweltbundesamt

Pesticides are usually measured in the surface waters of streams but rarely in sediments. However, several studies have found high concentrations of many pesticides in sediments compared to overlying waters. Some of these pesticides such as organochlorines, organophosphates and synthetic pyrethroids are hydrophobic and are expected to bind to sediments; however, recent surveys of some streams in Victoria, Australia have detected hydrophilic pesticides such as the herbicide simazine and the fungicides myclobutanil and pyrimethanil at concentrations from 100 to 1000 times higher in sediments than in overlying waters. Sites where this occurred were typically located downstream of intensive horticulture operations with a history of heavy pesticide use. This suggests that the elevated concentrations in sediment may indicate recent acute pollution events, and that sediments may act as passive samplers for hydrophilic pesticides more generally. We tested this hypothesis by conducting an experiment in two 100 m long artificial streams where we spiked the surface waters with high concentrations of the pesticides herbicides simazine (30 μg/L) and atrazine (30 μg/L), the neoninoid insecticide imidacloprid (50 μg/L), and the fungicides myclobutanil (100 μg/L) and pyrimethanil (100 μg/L). The sediments consisted mostly of fine sands with the exception of four 2m long blocks in each stream that contained fine sediments. The carbon content was 0.9% in half of these blocks and 0.1% in the other half. The surface water was replaced by clean water 12 hours after spiking to simulate a spray event, and the pesticide concentrations were then monitored over the next 28 days in surface waters and sediments. Pesticide concentrations remained low in the surface waters over this period, however, remained elevated in sediments for several weeks following the initial spiking. This experiment confirms that sediments can act as effective passive samplers for some pesticides and could serve as potential indicators of recent acute pollution events.

WEI19
The Guadiana River Estuary salinization, an opportunity for traditional sea salt production recovery

N. Sainz, Universidad de Cadiz and Universidad del Algarve; T. Boski, University of Porto / Laboratory of Geochemistry, School of Science and Technology, University of Porto

This study evaluates how changes in the volume of freshwater input due to dams' construction affect water salinity downstream and how this can induce changes in sea salt production and socio-economy in the Guadiana estuary, which is a natural southern border between Spain and Portugal. Fluvial discharge of Guadiana has changed over the past few decades, since river flow has been substantially reduced by the construction of two hydropower dams in the river basin, the most recent being the Alqueva dam (2002). Mean monthly river flow used to vary markedly on a seasonal and yearly basis, but a sharp increase in salinity in the river mouth has been reported. In 1959, the tidal channel of Leziria of Castro Marim Municipality (Portugal) was supplying water to salt works with an average salinity of approximately 21.5‰, and after the closure of the last dam the same channel can reach a salinity of 30‰ during the salt production period. The observed salinization in the Guadiana estuary applied to a recovery of the abandoned traditional salt production in the area which would cause an increase of 46.3% in the salt produced at the same land surface, and assuming the same evaporation rate for both levels of initial salinity. Although researchers have linked salinity and changes in sea salt production to the ecological changes detrimental to the estuarine environmental quality, the creation of employment on the Portuguese Guadiana River basin could benefit from the salinization of the lower estuary with the recovery of abandoned traditional salt works. Active salt works removed the clean water 12 hours after spiking to simulate a spray event, and the pesticide concentrations were then monitored over the next 28 days in surface waters and sediments. Pesticide concentrations remained low in the surface waters over this period, however, remained elevated in sediments for several weeks following the initial spiking. This experiment confirms that sediments can act as effective passive samplers for some pesticides and could serve as potential indicators of recent acute pollution events.
Influence of a seawater pulse on the structure of freshwater invertebrate communities

C. Venclovič, Biology; C. Shinn, IMAR-Marine and Environmental Research Centre / Laboratoire Evolution et Diversité Biologique; M. Abello, Instituto Politécnico de Coimbra; M. Moreira-Santos, University of Coimbra, IMAR-CMA / Department of Life Sciences; J. Lopes, University of Aveiro / CESAM Biology Department; R. Ribeiro, University of Coimbra

Many coastal freshwater ecosystems are already facing seawater intrusions and recent projections, predicting a worst scenario of 80 cm rise by the end of 2100 suggest that, in a near future, these seawater intrusions in coastal ecosystems will, most probably, be intensified. Salinity is an important abiotic factor influencing communities’ abundance, structure and composition by, for example, influencing reproduction and growth of species that compose a community. Accordingly, the present work intended to evaluate the long-term effects of a pulse of seawater intrusion in the structure and composition of a freshwater invertebrate community simulated in outdoor mesocosms. Model outdoor mesocosms were pulsed-contaminated with the following independent salinity values: 0, 2.02, 3.34, 5.51, 9.09, and 15.0 mS cm⁻¹. Three distinct periods were established: seawater intrusion (33 days), start of recovery with periodic dilutions (RD, 38 days) and recovery from end of dilution onward (RDP, 126 days). The most common macroinvertebrate taxonomic groups, identified in the outdoor mesocosms, were Diptera, Ephemeroptera, Mollusca, and Odonata. Diptera showed the highest abundances in the control and at salinity 15, in the RDP. Mollusca (composed mainly by snails) appeared at salinities 9.1 and 15 mS cm⁻¹, only in RDP. Ephemeroptera showed the greatest levels of biodiversity at salinity 15 mS cm⁻¹ did not recover after SI. In zooplankton, groups presented at highest abundances were, in decreasing order, Rotifera, Copepoda, Branchiopoda and Ostracoda, during SI phase. As expected, after SI, a clear decrease in organism’s abundance (A) and species diversity (SD) was observed, with stronger effects at 15 mS cm⁻¹. The most representative groups during this period were Rotifera and Copepoda. During the RDP, a forecasted an increase in biodiversity, among the four groups. Increase of the Ostracoda density relatively to the RD was noticed only in C and 2.02 mesocosms. Plus, in the RDP, some fluctuations in both A and SD were observed. These fluctuations were also observed in control, suggesting that the communities exposed to different salt concentrations were returning to an initial equilibrium state, being, thus, a possible indication that the zooplankton community can recover after exposure to a seawater pulse.

WE121 Precipitation and Re-use of Chromium from Residual Water in Leather Industry, San Benito (Bogotá Colombia)

L. Cuervona, Universidad Nacional Autónoma de México / Veterinary School; N.E. Ortiz, Universidad Santo Tomás (Bogotá) / Facultad de Ingeniería Ambiental

ABSTRACT Technical and sustainable viability or chromium retrieval was evaluated in residual waters for a leather industrial process in San Benito (Bogotá); the previous work was achieved with precipitating reactions using 4M sodium hydroxide solutions and looking for the regeneration of the element, adding sodium sulphate to a lignin solution in which is regenerated by drugs, with the goal of chromium re-use, in the same process. The application of the results in this research process would contribute to minimize of water contamination with chromium, besides the economic expenses reduction. It started from a previous work it (Ortiz, 2013), where the waste water chromium content was reduced in this leather industry chemical process in 99.9%, from initial average of 10 g/L to lower levels, posing a serious threat to coastal freshwater ecosystems through increased salinity. It is urgent then to understand how organisms respond to changes in salinity. Here we investigate the multi-generational effects of increased salinity in Daphnia magna. Fitness measures were obtained across two generations from individuals allocated to five salinity treatments. The treatments represented a gradient in salinity, from a low salinity to a high level of salinity. We were also interested in examining the fitness response to uncertainty in salinity, as for a fifth treatment the level of salinity varied unpredictably between the low and high level of salinity. Salinity had a significant effect in decreasing the chronological and reproductive longevity and total reproduction. Surprisingly, individuals allocated to the variable salinity treatment showed the greatest decrease in fitness. Single species, such as Daphnia, may be better adapted to respond to mean changes in conditions than to variance in conditions. In terms of F1s, results showed that offspring produced by mothers exposed to constant levels of salinity lived longer and produced more F2s, than offspring produced by mothers allocated to the variable salinity treatment. The effects of variability in salinity were also greater in later broods (7th) than in earlier broods, suggesting that, prolonged exposure of mothers to fluctuating salt stress resulted in offspring’s with less ability to cope with salt stress. In combination our results highlight the importance of integrating multi-generational effects when performing prospective risk assessments of increased salinity to freshwater ecosystems, aiming at an accurate protection of these ecosystems.

WE124 Effects of stream salinization on fungal-mediated leaf processing

C. Canhoto, Life Sciences; J. Silva, CEF & University of Coimbra / Life Sciences; S. Silva, University of Porto / IPATIMUP, Faculty of Sciences; A. Lirio, A. Gonçalves, CEF & University of Coimbra; L. Guilhermino, CIIMAR University of Porto; F. Bärlocher, Mount Allison University

Salinization of streams and rivers is a growing concern all over the world. Information about the potential consequences of this threat on the functioning of streams is scarce particularly on what concerns key ecosystem-process levels such as leaves decomposition. In this study we evaluated the effects of a gradient of NaCl (maximum concentration: 0-16 g/L) on fungal growth, sporulation, leaf litter (oxygen consumption - respiratory) decomposition by single species and associated microbial respiration. We used nine species of aquatic lignophytes common in Portuguese streams - Anguillula porifera, Actinoporus tetracadul, Clavariospis aquatic, Flagellella curvula, Heliscus lugdunensis, Lemmoniera pseudofusca, Tetrachaeum elegans, Tetracadium marchalianum, Tricladium chaeoalium. Fungal growth, evaluated as mycelium diameter, was inhibited in all tested species, in decreasing order, NaCl ≥ 8g/L, NaCl ≥ 4g/L, NaCl ≥ 2g/L and NaCl ≤ 0g/L. Sporulation, assayed at three different concentrations of NaCl, showed significant inhibition at NaCl ≥ 8g/L. Concentration of NaCl negatively affected both parameters in all cases. Although tests with fungal assemblages are needed, results suggest that streams salinization (NaCl ≥ 2g/L) may affect fungal diversity, leaves incorporation into secondary production and nutrient cycling.
Retinoid-like compounds produced by phytoplanктон

J. Prieborgová, Masaryk University, Faculty of Science, RECETOX, E. Sychrova, RECETOX Research Centre for Toxic Compounds in the Environment; M. Smutna, K. Novakova, Masaryk University / Faculty of Science RECETOX; K. Hiekel, DESMA/University of Würzburg, Faculty of Science, RECETOX / RECETOX Research Centre for Toxic Compounds in the Environment

The use of pesticides that disrupt the functioning of endocrine systems is a growing concern, as retinoid-like compounds produced by phytoplankton may interfere with the signaling of retinoids in different ecosystems. Retinoids play a crucial role in vertebrate development, influencing cell proliferation, differentiation, and morphogenesis. Animal studies have shown that retinoids can be affected by environmental pollutants. The aim of our work was to evaluate the ability of phytoplankton to produce retinoid-like compounds, which could be released into the environment. The study included various cyanobacteria and algae representing different orders. We tested in vitro the potential of mixtures of biologically active compounds from laboratory-cultured phytoplankton species to induce responses mediated through retinoid acid receptor (RAR) using murine embryonic carcinoma cell line luciferase reporter, which contains reporter luciferase gene under the control of retinoic acid-responsive element. Extraction process was optimized for maximal yield of bioactive compounds from studied species. We found activation of retinoid receptor after exposure to intracellular and extracellular samples from different species including both cyanobacteria and algae. Variability of production of retinoid-like compounds including also the influence of light conditions was characterized in more detail for selected species, which are frequently occurring at high abundances in the fresh water environments. The detailed investigations focused on one coccal (Microcystis aeruginosa) and one filamentous cyanobacteria (Anabaenomenon gracile) and an algae (Desmodesmus quadricauda). The extracts of the three selected species showed mean retinoid-like activity in the range of 705–3098 ng ATRA/g dry weight. In case of exudates, mean retinoid-like activity was in range of 96–779 ng ATRA/L for the cyanobacteria, while the exudates of the alga Desmodesmus quadricauda showed no detectable activity. Our study underlines the availability of various species of both cyanobacteria and algae to produce retinoid-like compounds. They could be most important and relevant in aquatic ecosystems with high abundance of retinoid-producing species, e.g., in case of massive water blooms especially in shallow water bodies. The research was supported by the Czech National Science Foundation grant No. P503/12/0553.

Soil Ecology and Ecotoxicology: Pillars Supporting the Conservation and Protection of Soil Biodiversity and Ecosystem Services during the International Year of Soils (P)

Pesticide risk assessment for soil living organisms: the present and the future

P. Sousa, BMARCMA / Department of Life Sciences University of Coimbra; M. Amorena, EFSA - Institute for Plant Production Sciences; D. Autieri, Autieri / Pesticides Unit; A. Ippolito, EFSA - European Food Safety Authority / Institute for Environment and Sustainability IES; R. Sharp, EFSA - European Food Safety Authority / Pesticides Unit; F. Streissl, EFSA - Pesticides Unit; C. Szentes, Pesticides Unit

In view of the revision of the current terrestrial guidance document (European Year of Soils (P) SETAC Europe 25th Annual Meeting Abstract Book 2014), we will discuss the importance of risk assessments for soil arthropods. Thus, the question arises whether the risk assessment for soil arthropods or the product is applied directly on or deeper in soil are affected by topically applied a.s. which predominantly remains in the top centimeter during the entire experiment. Hence, linking effects and exposure through the soil profile will not be simply straightforward. Substances applying on soils will always display highest concentrations immediately after application and in the upper centimeter of the soil. In our opinion, ERA for soil organisms exposed to PPP should therefore consider in a first step the upper first soil centimeter as the relevant depth to describe a realistic worst case independently from the organisms’ home range. The initial conditions soil organisms are exposed to in the field are a pronounced vertical stratification of the a.s. concentrations and not a constant concentration over the whole depth. It is our assumption that, if the concentration in the upper soil layer is high enough to elicit effects, a relevant share of the soil bioavailability will be exposed to it either because they live principally in the upper centimeters or because they move in the soil profile. In a refinement step, numerical models are considered suitable tools to estimate the distribution of a.s. in soil profiles over relevant time frames depending on their fate properties. The soil layer containing the largest proportion of a.s. after a given time might indicate the relevant depth to calculate the PEC. Such refinements might be suitable to address questions of recovery or recolonisation of soil organisms after initial effects due to PPP use. Toschki et al., 2014; Evaluation of the risk for soil organisms under real life condition, presentation 350, SETAC Europe 24th Annual Meeting, Basel, 15.05.2014

Which is the relevant soil depth to assess the risk for soil organisms exposed to PPP?

S. Eeber, Umweltbundesamt; P. Kotschik, D. Felsmann, UBA Umweltbundesamt; W. Koenig, Federal Environment Agency (UBA); S. Pieper, German Federal Environment Agency (UBA) / Plant Protection Products

Recent concepts regarding the environmental risk assessment (ERA) for soil organisms exposed to plant protection products (PPP) suggest that relevant soil depths to calculate predicted environmental concentrations (PEC) should relate to so-called ecological relevant soil depths typical for the vertical home range of different soil organism groups (e.g. EFSA Journal 2010:8(10):1820). The hypothesis that the toxic effects of active substances (a.s.) relate to the typical vertical living range of organisms and therefore are captured by a mean PEC for the entire horizon has not been substantiated up to now. To support further discussion, several data on vertical distribution of animals and a.s. in the soil profile are presented here. Toschki et al. (2014), for example, report that soil organisms living deeper in soil are affected by topically applied a.s. which predominantly remained in the top centimeter during the entire experiment. Hence, linking effects and exposure through the soil profile will not be simply straightforward. Substances applying on soils will always display highest concentrations immediately after application and in the upper centimeter of the soil. In our opinion, ERA for soil organisms exposed to PPP should therefore consider in a first step the upper first soil centimeter as the relevant depth to describe a realistic worst case independently from the organisms’ home range. The initial conditions soil organisms are exposed to in the field are a pronounced vertical stratification of the a.s. concentrations and not a constant concentration over the whole depth. It is our assumption that, if the concentration in the upper soil layer is high enough to elicit effects, a relevant share of the soil bioavailability will be exposed to it either because they live principally in the upper centimeters or because they move in the soil profile. In a refinement step, numerical models are considered suitable tools to estimate the distribution of a.s. in soil profiles over relevant time frames depending on their fate properties. The soil layer containing the largest proportion of a.s. after a given time might indicate the relevant depth to calculate the PEC. Such refinements might be suitable to address questions of recovery or recolonisation of soil organisms after initial effects due to PPP use. Toschki et al., 2014; Evaluation of the risk for soil organisms under real life condition, presentation 350, SETAC Europe 24th Annual Meeting, Basel, 15.05.2014

Is the risk for soil arthropods covered under the current ERA scheme?

D. Ruf, Agroscope Changins-Wädenswil; E. Kohlschmid, O. Daniel, Agroscope / Institute for Plant Production Sciences IIPS

Testing effects on earthworms and terrestrial non-target arthropods (leaf dwelling species) is an integral part of the ecotoxicological risk assessment for the authorisation of plant protection products. In contrast, testing of effects on soil arthropods is only required if the risk assessment of foliar applications raises concerns regarding non-target arthropods or the product is applied directly on or into the soil (Commission regulation (EU) 283/2013). It has been shown that soil arthropods are substantially more sensitive to several active ingredients (insecticides, fungicides and herbicides) than earthworms, indicating that the earthworm based risk assessment for soil organisms might underestimate the risk for soil arthropods. Thus, the question arises whether the risk assessment for non-target arthropods would trigger the testing of effects on soil arthropods in such cases. To answer this question a survey of information available in the Swiss ecotoxicological database, EFSA conclusions on pesticides and the open literature is conducted. The results will be presented and further needs for risk evaluation on soil arthropods are discussed.

An ecosystem services framework for revealing trade-offs in crop production


WE125

WE128

WE129
the potential consequences of the EU decision to restrict neonicotinoid use and the review of authorisations under EU pesticide regulations on winter wheat production. Impacts of the most commonly used pesticides for winter wheat and associated break crops were evaluated by assessing: hazard, need for risk mitigation, and risk mitigation measures required. The consequent direct impacts on ecosystem services were estimated. We found that restricting pesticide use can protect the production of multiple ecosystem services, however, tighter restrictions would have to be implemented significantly to restore the viability of farm businesses, food security in the UK, EU and potentially globally. Hard choices need to be made about which ecosystem services we enhance where, for the benefits we consider most critical.

WEI30 Getting Rid of Formalin: Using AITC and Mustard Powder Preparations for Earthworm Extraction in Combination with Handsorting

T. Volmer, Eurofins Agrosciences Europe GmbH / Field Ecotoxicology; O. Klein, S. Knaebe, EAS EcoChem GmbH / Ecotox Field Currently, an ad-hoc group led by the German Federal Environmental Agency is working to transform a long-lasting earthworm bioassay revealing potential for intrinsic recovery of Collembola. Thus, the toxicity did not decrease remarkably in the 2nd bioassay after aging of the test substance in soil. Case studies indicated that the test system determines whether a population which hatched under impact of a test substance in a modified OECD 232 bioassay were introduced into a second consecutive bioassay containing the aged test substance in soil. This test system determines whether a population which was initially impacted by pollutants on earthworms (into an OECD guideline. This transformation process provides the possibility to generally adapt the method to the latest developments and requirements. One of the topics under discussion is the choice of the chemical for extracting deep-burrowing earthworm species from soil, as the present standard extraction fluid formalin is known to be hazardous to humans and to the environment and should therefore be replaced by an alternative extracting agent with more favourable properties. Mustard powder (ground seeds of Sinapis alba L.) and AITC (Allyl Isothiocyanate) have already been proposed by several authors (e.g. Zaborski (2003) and Bartlett et al. (2006)) as alternative expellants with mixed results when applied directly to the soil surface. We present results from field experiments conducted in 2010-2014. The overall aim was to compare the efficiency of aqueous solutions of mustard powder of different preparations and of AITC in extracting earthworms from the soil below hand-sorting holes to that of a formalin solution as described in the current guideline. Earthworm extractions were performed at 8 different sites, all located in southern Germany and differing with respect to soil characteristics (pH, soil texture), land use (arable, grassland) and earthworm populations. Each site was sampled one to three times during periods of high earthworm activity. At each site and sampling occasion, 4 to 6 replicates (holes of 0.25 m², 0.2 m deep) per expellant were sampled. Our results indicate that AITC and mustard powder are suitable replacements of formalin for the extraction of deep-burrowing earthworms from below hand-sorting holes. Pro and cons of the expellants will be discussed with respect to the extraction efficiency, practicability of use in the field, and user- and environmental safety considerations. References Bartlett, M. D., Harris, J. A., James, I. T. & Ritz, K. (2006): Inefficiency of mustard extraction technique for assessing size and structure of earthworm communities in UK pasture, Soil Biol. and Biochem. 38(9), 2990 – 2992. Zaborski, E. R. (2003): Allyl isothiocyanate: an alternative chemical expellant for sampling earthworms, App. Soil Ecol. 22(1), 87 – 95.

WEI31 Assessing the potential for intrinsic recovery in a Collembola two-generation study - possible implementation in a tiered soil risk assessment approach for plant protection products

G. Zielke, Bayer CropScience AG / Ecotoxicology; P. Kabouf, BASF SE; M. Barth, BioChemagrar GmbH; U. Frommholz, Bayer CropScience; S. Royer, BASF SE; Global Ecotoxicology, Crop protection; S. Friedrich, BioChem agrar GmbH A Collembola two-generation study was developed in order to assess the potential for intrinsic recovery within an intermediate tiered collembola risk assessment approach for plant protection products. Juvenile individuals of Folsomia candida hatched under impact of a test substance in a modified OECD 232 bioassay were introduced into a second consecutive bioassay containing the aged test substance in soil. This test system determines whether a population which was initially impacted by a substance in a 1st bioassay shows normal reproduction and survival in a 2nd bioassay after aging of the test substance in soil. Case studies indicated that the collembola two-generation study is a sensitive test system. After an initial effect on F. candida in the 1st bioassay it depends on the properties of the substance whether the toxicity is reduced in the 2nd bioassay. In case persistent substances were applied the toxicity did not decrease remarkably in the 2nd bioassay. Contrastingly, fast degrading or dissipating substances were shown being of reduced toxicity in the 2nd bioassay revealing potential for intrinsic recovery of Collembola. Thus, the collembola two-generation study is able to differentiate between substances showing a potential long-term effect and substances causing only short term effects. Comparing the results of the two-generation studies with effect data from semi-field and field studies indicates a high degree of conservatism using the collembola two-generation study as an intermediate tiered test system. This approach represents a valuable and efficient tool for the soil risk assessment providing an intermediate tiered refinement option for highly conservative tier 1 collembola risk assessment.

WEI32 Natural variation of Collembola abundances on agricultural sites

P. Kabouf, BASF SE; J. Lueckmann, M. Cartay, M. Faupel, Rifcon GmbH Collembola field studies are frequently conducted as part of the risk assessment of plant protection products (PPPs). Currently no guideline and exact assessment criteria are available, making meaningful evaluation of these studies difficult. Collembola communities are typically highly variable in their abundance and composition; therefore interpreting whether or not, after application of a PPP, a risk is induced is challenging. In order to support evaluation of potential PPP effects on Collembola communities we present the results of five field trials conducted with PPPs. The variations of abundances (based on the relative standard deviation of mean abundance) at two different spatial levels were determined at the species level. Results show variations up to a 3.5-fold of mean abundance (for Isotomurus palustris) with most species varying by a factor of 1.5 - 2.5. Mesaphorura critica showed the least variation, i.e. a 1.4-fold of its mean abundance. Specific species differences were observed for both low and highly abundant taxa. These differences should be considered when interpreting Collembola field data. Overall, Mesaphorura krausbaueri appeared to be an appropriate representative species for field studies in the context of PPP registration as it was found at all test sites in relatively high abundances and showed relatively low variability in comparison to other species.

WEI33 Field monitoring of soil mesofauna in conventional (chlorpyrifos-treated) and organic Cider-Apple Orchards in Herefordshire UK: Differences in Collembolans, Mites and Enchytraeids

T. Hamburgh, IBACON GmbH / Terrestrial Ecotoxicology; S. Norman, RidgewayEco; N. N. Poletika, Dow Agro Sciences LLC / Field Exposure and Effects Department The influence of chlorpyrifos on diversity and abundance of soil mesofauna (Collembola, mites, enchytraeids) was monitored for two years. 3 conventionally-managed (chlorpyrifos-treated) cider-apple orchards were chosen as trial sites. In conventional orchards, we measured earthworm populations and field margins at the edges of the conventional orchards served as ‘references’. These references are used for general comparison with the numbers of sampled organisms from within the conventionally-managed orchards. All trial sites were located in Herefordshire, a major cider-apple growing area in England. Chlorpyrifos was applied bi-annually, for the control only of apple blossom weevil and sawfly (both impact apple yield). 12 soil organism samplings were carried out between April 2013 and April 2015. In the conventional orchards all trees were arranged in rows with defined distances within and between the tree rows, separated by ‘tramlines’. These tramlines were intensively driven-on by tractors during plant protection and maintenance measures. In organic orchards the employment of large machines was less intensive and clear tramlines were missing. Moreover, tree density was lower and 2 orchards were extensively grazed. 2 types of sampling areas were established in each orchard: tramline and tree row. Additionally, only in the conventional orchards, field margins, which were located near hedges that served as borderslines were used as a third type. Heat extraction of soil cores in a Mac Fayden apparatus and funnel pitfall traps were used to sample eudaphic and (hem)edaphic collembola, soil mites and enchytraeids (found at only 1 of 5 sites) showed the least variation, i.e. 2.5. Isotomurus palustris showed the greatest variation, i.e. 1.4-fold of its mean abundance. Soil organism diversity gradient were created using a dilution to extinction approach. Mesaphorura krausbaueri was chosen as a representative species for field studies in the context of PPP registration as it was found at all test sites in relatively high abundances and showed relatively low variability in comparison to other species.
mesocosms had reached similar levels of bioactivity. OECD307-compliant aerobic soil degradation studies of four model pesticides (2,4-D, Terbutylazine, Azoxyuron, Bicyclopyrone) were conducted using these equilibrated soils. Analysis of compound degradation rates revealed pesticide-specific sensitivity to microbial erosion as well as clear differences in the patterns of response. The microbial 2,4-D degradation function was robust up to 10^6 dilution, but was disrupted in highly enriched systems. Conversely, Azoxyuron half-life was maintained beyond (>417 days) in all diluted mesocosms compared to the ‘fresh control’ DT50 of 77 days, indicating a high dependency on microbial diversity. Degradation of Terbutylazine did not follow the trend (more erosion = longer half-life/lower rate) observed for 2,4-D and Azoxyuron. Data so far indicates that this test system is suitable for studying the impact of microbial diversity erosion on pesticide biodegradation function. Our findings suggest that structurally different chemicals respond differently to microbial diversity erosion. Differences in microbial diversity may therefore explain, at least in part, soil-to-soil variation in the field and changes in microbial diversity occurring during processing of soils for lab studies may therefor explain some of the lab-to-field variation frequently observed.

WE135

Diploponds as soil bioindicators: morphological alterations in the midgut cells of Rhinocricus padbergi exposed to a metallo-insecticide

R.B. Souza, Sao Paulo State University - UNESP / Biology; C.M. Sousa, Sao Paulo State University - UNESP / Biology; J.B. Fernandes, Universidade Federal de São Carlos / Theses; R.C. Ocasio, Universidade de São Paulo / Centro de Estudos de Insetos Sociais; C. Fontanetti, Sao Paulo State University - UNESP / Biology

Keywords: histopathology; histology; millipedes; soil ecotoxicology Diploponds play an important role in the dynamic of soils, where they occupy the trophic level of decomposers, since they promote its aeration and enrichment. Due to their habits, scientists have discussed their indicators in soil ecotoxicological studies, using midgut histopathology as a biomarker. This technique enables diagnosing cellular and subcellular symptoms resulting from intoxication, as well as symptoms of cellular death. It can also reveal reactions in response to chronic and sublethal exposures in cells and tissues. Nowadays, the indiscriminate use of pesticides in soil is one of the main environmental problems. The most efficient active ingredient to ant control is sulfuramid, but it was prohibited by the Stockholm Convention due to its toxicity. As an alternative, researchers have developed a metallo-insecticide, whose formula consists in a natural product complexed with an inorganic metal. Thus, the aim of this study was to analyze morphological alterations in the midgut cells of diplopond Rhinocricus padbergi induced by the metallo-insecticide Mg6g[Mg(5-methyl-phen)hesperitin](H2O)3(2CH3COO). Three treatments were performed: control, using soil from the local where the animals were collected, and two treatments containing metallic-insecticide at the concentrations of 0.5 mg/mL e 1.0 mg/mL. Nine animals were placed in each treatment. After 21 days, 3 animals from each treatment were dissected and the midgut was prepared for histological routines (hierarchies). After the histological routine, the histopathological alterations were analyzed semi-quantitatively for each diplopond. The data obtained in histopathological evaluation were compared to the results obtained for the individuals exposed to the control soil sample with the Kruskal Wallis test and p < 0.05. Increase in epithelial turnover, release of secretion vesicles and presence of granules in hepatocyte cells were observed in animals exposed at both concentrations. However, none of these alterations were statistically significant. Therefore, the metallo-insecticide was not toxic to R. padbergi. Financial Support: FAPESP (2012/2019-5)

WE136

Ecotoxicological assessment of the effects of agrochemicals on non-target organisms

S. Curriesc; m. saenz, Universidad Nacional de Lujan CONICET; J. Alberdi, UNLU; W.D. Di Marizio, Universidad Nacional de Lujan-CONICET

Increasing world use of synthetic herbicides and insecticides to sustain high – yielding crop varieties may affect non target organisms. The pampas region of Argentina has high biodiversity and eco systems. The aim of present study was to evaluate the usefulness of a series of endpoints for assessing adverse effects of the extensive use of agrochemicals. The soils were sprayed with commercial chlorpyrifos or glyphosate at manufacturers ‘recommended rate. Also, soils of extensive fields were analyzed. Water elutions of contaminated soils were performing for determine agrochemical mobility in the environment. Biomass growth, level of lipid peroxidation, antioxidant stress enzymes (catalase and peroxidase) in Vicia faba roots, and genotoxicity in coelomocytes of Esinia fetida were assayed for aqueous elutions evaluation. Root elongation, seed germination in Lactuca sativa and genotoxicity in coelomocytes of Esinia fetida were performed for soils evaluation. The results show both agrochemicals exert toxic effects in assayed species. The

agrochemicals induce phytotoxity in Lactuca sativa seeds, stimulate activity enzymatic in Vicia faba roots, and increase levels of DNA damage in coelomocytes exposed to ex situ or in vivo conditions. In the present work a level of bioassays and biomarkers has been assessed on soils treated with glyphosate and chlorpyrifos. This study show dangerous effects in several organisms of glyphosate and chlorpyrifos formulations when applied at the recommended concentrations. The results indicate intensive use of both agrochemicals in crops could represent a risk for soil biota.

WE137

Assessing the ecotoxicity of gold mine tailings utilizing earthworm and microbial assays

M. Mahboub, C. Van Coller-Myburg, North-West University

Problems associated with mining is the disposal of wastes on tailings disposal facilities (TDF) resulting in possible soil contamination. Earthworm and microbial assays can be utilized to assess the ecotoxicity of contaminants. The aim of this study was to determine the ecotoxicity of gold mine tailings by using earthworm (Eisenia andrei) bioassays, neutral red retention time analysis (NRRT) and enzymatic analyses. Endpoints included changes in biomass, reproduction, mortality, lysosomal membrane stability (NRRT), tissue metal concentrations, dehydrogenase activity, bg-glucosidase, urease, acidic phosphatase and alkaline phosphatase analyses. Results indicated high concentrations of Ni in the material as well as bioaccumulation of lead and arsenic in the earthworm body tissue after exposure. Enzymatic activity was higher in the revegetated tailings than in the un-renatured tailings. Both bioassays and NRRT and including areas have an acidic pH which affects earthworms and metal bioavailability. Soil enzymatic activities in reference soils were significantly higher compared to those of TDFs making a sensitive indicator of metal pollution in mining areas. Growth, reproduction and lysosomal membrane stability of earthworms has also been shown to be sensitive endpoints to assess the ecotoxic effects of gold TDFs.

WE138

Application of a single species and microbial test method suite for the assessment of copper substances in soil

S. Kvas, Environment Canada / Biological Methods; E. Ritchie, C. Fraser, H. Lemieneux, A. Crossman, M. Lazarus, R. Subasinghe, Environment Canada / Biological Assessment and Standardization Section; J. Prince, Environment Canada / Biological Assessment and Standardization Section; L. Beaudette, Environment Canada / Ecotoxicology and Wildlife Health Division; R.P. Scroggins, Environment Canada / Biological Methods

Scoria, fauna and microorganisms are important components of a healthy soil. As such, a multi-testing approach involving each component is required to best estimate the risk associated with contaminants in soil. To evaluate the impact of two copper substances (copper oxide (CuO) and copper sulfate (CuSO4)) in a sandy loam field-collected soil, a series of single species and microbial tests were applied. Effects on plant (red clover and northern wheatgrass) growth and invertebrate (Eisenia andrei and Folsomia candida) survival and reproduction were evaluated. Microbial assays (heterotrophic plate count and fumigation extraction), activity (organic matter decomposition, nitrification, and respiration) and community structure (enzyme assays, community level physiological profiling, and DNA sequencing) followed by data integration of each microbial test. To ascertain the effect of copper in each test, total and ionic (e.g., using an ion selective electrode) soil copper were measured. This comparison may be useful in future copper substance toxicity research where only the ionic portion will be measured.

WE139

Estimation of soil health index using metal-remediated soil

S. Kim; J. Kwak; S. Nam; R. Cui, Y. Chae, J. Moon, Konkuk University; S. Jeong, Kunsan National University / Dept of Environmental Engineering; Y. An, Konkuk University / Department of Environmental Science

Soil health index (SHI) was proposed to evaluate the soil health before and after remediation. SHI was based on the standard process including 1) determination of assessment factor (AF), 2) indicator classification, 3) indicator ranking and weighting, 4) multi-criteria calculation and 5) determination of re-assessment conditions. The soil function related AFs were determined as soil productivity (SP), soil sustainability (SS), biodiversity (Bd), and risk (Ri). Soil productivity assessment indicators including physical stability (aggregate stability, bulk density, texture, water relations (infiltration, water holding capacity), nutrient cycling (cation exchange capacity, organic matter, total nitrogen, pH, total phosphorus, enzyme activity), and soil physical quality (plant growth, photosynthetic capacity)). Soil stability includes the physical (aggregate stability, bulk density, texture, water holding capacity), chemical (cation exchange capacity, organic matter, pH), and biological stability (earthworm activity, total glomalin related soil protein, enzyme activity). The indicators for biodiversity evaluation were chosen as earthworm, microbial biomass C, nematode, plant, soil algae, and springtail diversities, and the soil contamination concerns standard and bioaccumulation were assessed for human and ecological risk evaluation. Indicator ranking and weighting of each AF was determined using principal component analysis (PCA) and factor analysis (FA) method. The total data set of indicators in each AF was scored using standard scoring functions (more-is-better, less-is-better, and optimum), and soil health indexes of SS, Bd, Ri 361
calculated by integration of each weighted indicator score. Final SHI was determined using sum of each weighted SHI. This subject is supported by Korea Ministry of Environment as the GAIA project (2014000560001).

WE140 Evaluation of algal inoculation method for assessing soil algal toxicity S. Nam; Y. An, Konkuk University / Department of Environmental Science To expand the ecological diversity of terrestrial toxicity data, it is needed to focus on soil algae which act as a food source for many microfauna and mesofauna. However, there were no international guidelines for assessing soil algal toxicity. Especially, algal inoculation position is directly related to the efficient use of light for growth. In this study, we assessed the optimal inoculation method of soil algae in soil media. OECD and Lufa 2.2 soils were used as test soils. Chlorococcum infusium of exponentially grown culture was used as a test species. To evaluate applicability of optimal algal inoculation, copper and nickel were used as test chemicals. Algal chlorophyll fluorescence was assessed after solvent extraction. Chlorophyll fluorescence of C. infusium was decreased with the elevated concentrations of copper and nickel. In conclusion, we verified that optimal algal inoculation method was applicable for assessing soil algal toxicity. This subject is supported by Korea Ministry of Environment as the GAIA project (2014000560001).

WE141 Effect of fluorine on the activities of soil exoenzymes Y. Chea, Konkuk University; H. Yoon, Korea Basic Science Institute; Y. An, Konkuk University / Department of Environmental Science Fluorine is one of the halogen elements which has great reactivity and electro negative. It is known to rarely combine with organic matters and combine strongly with clays. This study evaluated the toxicity of fluorine based on the activities of soil exoenzymes including acid phosphatase, arylsulfatase, fluorescein diacetate hydrolase and urease. Test soil harvested in Konkuk University was 24h sieved and air dried for 7 days before use. Sodium fluoride (NaF) was test material because it generally used for fluorine toxicity test. Blank soil were autoclaved for 20 minutes at 121°C and treated with 10% formaldehyde. Soil exoenzyme activities were measured in 0, 3 and 10 days using method of endpoint photometric with optical density measurement. As a result, there were concentration-dependent decrease of soil exoenzyme activities in acid phosphatase and arylsulfatase assay and insignificant decrease of soil exoenzyme activities in fluorescein diacetate hydrolase and urease. In conclusion, fluorine affected negatively on phosphorus and sulfur cycles in soil. This subject is supported by Korea Ministry of Environment as the GAIA project (2013000530001).

WE142 A one-year assessment of biochar effects on key soil invertebrates in a field trial under viticulture M. Prodana, University of Aveiro & CESAM / Department of Biology; S. Loureiro, Universidade de Aveiro / Biology; A. Amaro, University of Aveiro / Department of Biology and CESAM; D.N. Nunes Cardoso, CESAM, University of Aveiro / Department of Biology and CESAM Centre for Environmental and Marine Studies; R. Morgado, University of Aveiro / Department of Biology and CESAM; D. Verheijen, D. Keizer, University of Aveiro / Department of Environment and Planning CESAM; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; M. Santos, CESAM & Dept.Biology / Department of Biology and CESAM A. Bastos, University of Aveiro / Konkuk University / Department of Biology Centre for Environmental and Marine Studies CESAM The application of pinewood biochar (produced at 620ºC) to viticulture soils for reducing drought stress in vineyards is the focus of an on-going interdisciplinary field trial, in the wine-producing region of Bairrada (Central Portugal). The main aim of the present study was to monitor the long-term effects of large-scale biochar application on soil invertebrates, their function and structure. For that, survival and reproduction of the collembolan Folsomia candida and the feeding activity of the isopod Porcellionides pruinatus were assessed at time 0, 6 and 12 months, after soil amendment with biochar (4 and 40 t/ha) and biochar mixed with organic waste compost (40 t/ha). No significant differences between treatments and sampling times were observed regarding food consumption of P. pruinatus. Moreover, biochar and/or biochar-compost application did not affect survival or the number of produced eggs by F. candida. This suggests that pinewood biochar applied to calcareous clay soil under a temperate climate regime up to 40 t/ha, alone or mixed with compost, poses no-to-low risk to representative soil invertebrates, over a 12 month period. By combining two primary non-target organisms and structural and functional endpoints, the large-scale biochar amendment can influence soil biota. These results therefore, provide important information and complement physico-chemical parameters for assessing changes in soil quality over time, due to biochar application. Key words: biochar, soil, vineyards, ecotoxicity

WE143 Evidence of in vivo cytotoxicity of silver nanowires in earthworms via ingestion exposure J. Kwak; Y. An, Konkuk University / Department of Environmental Science Although earthworms are key species in the terrestrial ecosystem, researches on the toxicity of silver nanowires to earthworm species are not reported yet. In the present study, we investigated the acute soil toxicity and in vivo cytotoxicity of silver nanowires having two different lengths, then confirmed that in vivo cytotoxicities were highly related to ingestion exposure than dermal exposure route. In the individual-level, there are negligible effects of silver nanowires on survival, morphological abnormalities, pathological abnormalities and burrowing behaviors whereas the worms were died to silver nanowires in soil. Otherwise, toxicity trends of inhibition of intracellular testis activities in in vivo oxidative stress in the earthworm coelomocytes were analyzed by flow cytometer. In addition, we observed that shorter silver nanowires were more accumulated in the intestines than skin tissues. The results showed that the in vivo cytotoxicity could be mainly related to the ingestion exposure route in the earthworm species. Acknowledgement - This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future Planning (2014-R1A2A11050515).

Effects and accumulation process of organic pollutants and metals in bacteria, phytoplanктon and zooplankton (P)

WE144 Growth and physiological effects in Ankistrodesmus falcatus and Microcystis aeruginosa exposed to nickel stress J. B. Martínez-Ruíz, Instituto Politecnico Nacional / Escuela Nacional de Ciencias Biológicas Lab de Hidrobiologia Experimental; F. Martinez-Jerónimo, Escuela Nacional de Ciencias Biológicas-LP.N. / Laboratory of Experimental Hydrobiology In recent years the release of chemical pollutants to aquatic environments has increased, caused by anthropogenic activities. These pollutants produce acute and chronic effects in aquatic biota, affecting their growth, reproduction and development. The aquatic ecosystems are highly susceptible environments because they act as sink for toxic contaminants. Metals are one of the most common toxic contaminants released to the environment, Nickel is an essential metal who plays an important role in cellular physiology of some eukaryotes and prokaryotes. Some statistics reveal that nickel production worldwide is about 1.589 million tonnes. In Mexico, concentrations in water could be as high as 1.16 mg L⁻¹. Phytoplankion is an important community and is the main responsible of organic carbon production and oxygen (trough photosynthesis) as the basis of all the trophic webs in aquatic ecosystems, so the toxic effects produced at this level are expected to affect at higher trophic levels. In this study, two primary producers: the green microalga Ankistrodesmus falcatus and the toxicogenic cyanobacterium Microcystis aeruginosa were exposed to Ni⁰ stress in order to compare their responses. Both microorganisms were separately exposed to different concentrations of nickel, with continuous illumination at 27 °C. The cell density was daily quantified and, at the end of the essay, the protein (Lowry, 1951), lipids (Zöllner y Kirch, 1962), carbohydrates (Dubois et al., 1951) and photosynthetic pigments (Wellburn, 1994) were determined; for M. aeruginosa phycobiliproteins (Becker, 1994) and cyanotoxins concentration (Envirologix® kit) were also determined. Both primary producers were very sensitive to low concentrations of nickel, nevertheless the cyanobacterium was more sensitive to high concentrations of nickel. Nickel concentration in water exceeded the maximum limit so as to avoid toxic effects in the aquatic biota.

WE145 Toxicity Mechanisms Identification via Gene Set Enrichment Analysis of Time-Series Toxicogenomics Data: Impact of Time and Concentration C. Gao, Northeastern University / Bioengineering; D. Weisman, University Of Massachusetts Boston / Department of Biology; J. Lan, Civil Environmental Engineering; N. Gou, Northeastern University / Civil and Environmental Engineering; A. Gu, Department of Civil and Environmental Engineering; D. Weisman, University Of Massachusetts Boston / Department of Civil and Environmental Engineering Toxicity Mechanisms Identification via Gene Set Enrichment Analysis of Time-Series Toxicogenomics Data: Impact of Time and Concentration In recent years, reporter gene expression profiling of whole-cell bacterial arrays has been used for biosensing and real-time toxicity assessment. Bacterial stress responses are of a highly dynamic nature, and exhibit far more temporal complexity than simple dose-response shifts. Current high-throughput bioassays are able record real-time measurement over time and incorporate multiple experimental factors such as genes, conditions and dose concentrations. However, typical data analysis of these biosensors does not consider their temporal complexity, and therefore, discards valuable information that could potentially identify specific signaling pathways induced by the toxicant. To account for the dynamic response and identify perturbed pathways, this study applied our improved gene set enrichment analysis (GSEA), which is based on a novel time series common principal component analysis (CPCA). This method takes each gene’s temporal expression variation into consideration while determining differentially expressed set of genes. By examining the dynamic responses of reporter cells treated with various levels of
mimotycin C, hydrogen peroxide, or lead nitrate, the analysis identified enriched gene sets representing specific pathways, thereby suggesting different mechanisms of toxicity for these chemicals. In addition, we compared this time-sensitive analysis to a time-unaware GSEA and demonstrated the value of the temporal component. This study also investigated the dose dependency of the stress response activation profiles and discussed the impact of time and dose concentration on the gene enrichment analysis results. These results suggest the notion that whole-cell bioassays should account for the cells’ complex dynamic responses, thereby implying that both data acquisition and data analysis should look beyond simple traditional endpoint responses when designing high-throughput experiments in toxicogenomics studies.

**WE146**
Distribution character of organic micro-pollutants in fishes from classic schistosomiasis-endemic areas
G. Zhao, H. Zhou, China IWHR
Pentachlorophenol-Na (Na-PCP) was widely used to kill mullusk in these areas where schistosomiasis is epidemic. HCB has once been used to produce pentachlorophenol (PCP) and Na-PCP. Low-chlorinated phenols (CPs), Chlorobenzenes (CBs) and Polychlorinated biphenyls (PCBs) are the possible impurities of commercial Na-PCP, these organic micro-pollutants will be released into the environment with the use of Na-PCP. To investigate the pollution status of organic micro-pollutants in fish samples from classic schistosomiasis-endemic areas, 105 fish samples were collected. 17 CPs, 12 CBs and 28 PCBs were measured in fish samples using Varian CP-3800 GC/MS/MS technique. The results show that the contents of PCBs and PCP are 4.59 and 3.39 ng g⁻¹ dw in the samples, which were moderate and compared with the results reported in the other non-schistosomiasis-endemic areas. However, CBs were the predominant micro-pollutants (GM = 772.7 mg kg⁻¹ dw; n = 105), the concentration of CBs were significantly higher than those of reported in the other non-schistosomiasis-endemic areas. Dichlorobenzene (such as 1,2-Dichlorobenzene, 1,3-Dichlorobenzene and 1,4-Dichlorobenzene) are the most predominant congeners. The facts suggest there exists potential health risk due to the pollution of the CBs in these samples; therefore, more in-depth investigations of CBs contamination in these schistosomiasis-endemic areas are needed.

**WE147**
Coupling between phytoplankton and PCB dynamics revealed based on three decades of North Sea and Celtic Sea data
G. Evers, G. Senft, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; F. De Laender, Université de Namur ASBL / Laboratory of Environmental Toxicology and Aquatic Ecology; P. Gheysels, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology
GhEnToxLab unit
We analysed multidiscopal field observations (1979–2012) in the North Sea and Celtic Sea with additive models to infer spatiotemporal concentration trends of chlorobenzes (PCBs) and polychlorinated biphenyls (PCBs) for U.S. East Coast caught menhaden, the annual removal of PCBs from the Chesapeake Bay by the Commercial Menhaden Fishery Removal of Polychlorinated Biphenyls from the Chesapeake Bay by the Commercial Menhaden Fishery
J. Kalka, The Silesian University of Technology; E. Felis, The Silesian University of Technology; K. Kowalska, M. Nowrotek, J. Surmacz, The Silesian University of Technology

**WE149**
Predicting bioconcentration of emerging chemicals using model biomacromolecules
Y. Ruan, Nankai University, College of Environmental Science and Engineering; H. Sun, China Construction Bank
To understand bioconcentration is indispensable to evaluate the ecotoxic of a chemical. Lipid has long been recognized as the main constituent to bind organics, and Kow is used as an index to evaluate bioconcentration. However, recently, it has been found that lots of emerging chemicals are polarizable, and their bioconcentration can not be explained only by the partition into lipids. Binding to proteins is believed to exert great importance in bioconcentrate. Hence, it is necessary to develop new surrogates other than Kow to get a precise prediction on bioconcentration. In this study, the distribution coefficients (K) of 15 enantiomers of two kinds of chemicals —hexabromocyclododecanes (HBCDs) and β-blockers into several model biomacromolecules (three liposomes and two model proteins) were studied. Meanwhile, their bioconcentration into a microalgae (Spirulina platensis) was measured and compared with the model values. The correlation coefficients between the liposomes (Klip) and the proteins (Kprot) of the three HBCD stereomers had dissimilar results with their Kow, indicating some specific interactions other than partition being involved in the distribution into these model biomacromolecules. The Klip and Kprot of the four β-blockers were significantly affected by pH with a peak value around neutral pH due to the simultaneous change of their speciation and surface charge of the biomacromolecules. The K values the four β-blockers are of the same order as their Kow, however, their discrepancies were smaller than those of Kow. This indicates that for the hydrophobic β-blockers with low Kow, they could bind with the biomacromolecules through specific interactions. Raman and fluorescence spectroscopy were checked to get an insight into the microlevel mechanisms, which indicated that HBCDs and β-blockers interacted with the proteins through hydrophobic force and electrostatic interaction, respectively. Correlation between logBCF in the algae and logKow values revealed that Kow showed the best predicting capacity for all the 15 enantiomers. The low slope and high intercept in correlation equation indicates that Kow is not a good indicator for BCF. In all, bioconcentration of HBCDs was an integrated result of sorption by lipids and proteins as well as some other factors such as steric effects; while the binding to proteins played a main role in the bioconcentration of β-blockers.

**WE150**
Removal of Polychlorinated Biphenyls from the Chesapeake Bay by the Commercial Menhaden Fishery
J. Kalka, The Silesian University of Technology; E. Felis, The Silesian University of Technology; C. Haas, Academy of Health and Liberal Arts; R. Soroka, A. Sarno, Philadelphia University / College of Science Health and Liberal Arts; L. Zaoudeh, Academy of Natural Science and Engineering; D. Velinsky, Academy of Natural Science of Drexel University; J.E. Baker, University of Washington / Center for Urban Waters
The Chesapeake Bay is the largest estuary in the United States, receiving high nutrient inputs from various anthropogenic sources which fuel eutrophication within its waters. Concurrently, the estuary receives significant inputs of PCBs, largely from point sources from its industrialized and urbanized tributaries. Phytoplankton provide organic carbon rich sites for absorption/adsorption of PCBs and represent an important vector for PCB bioaccumulation within higher trophic level organisms such as zooplankton and planktivorous fishes. Atlantic menhaden (Brevortia tyrannus) are a key foraging fish in the Chesapeake Bay and near coastal regions. Acquired filters, juveniles and adults primarily feed on phytoplankton and zooplankton. By filtering vast quantities of planktonic organisms, these fish accumulate polychlorinated biphenyls (PCBs) from this pelagic dietary route. In the past decade, the commercial fishery for Atlantic menhaden landed an annual average of 144 kilotons of fish, of which an average of approximately 50% carry PCBs. Concurrently, efforts to eliminate PCBs from the Chesapeake menhaden fishery have reduced PCB concentrations in menhaden by 90% since 2006. This intense reduction fishery processes menhaden into fish oil and meal, high in omega-3 fatty acids. Using previously reported concentrations of PCBs for U.S. East Coast caught menhaden, the annual removal of PCBs from the Chesapeake Bay was estimated to be between 7 and 30 kg/year. In addition to export to the ocean, burial via sedimentation, and volatilization, removal of PCBs by commercial fishing of menhaden may represent a small, but intriguing loss pathway for PCBs to the Chesapeake Bay. Future estimates will include other commercial/recreational fisheries of species such as blue crab and striped bass. Additionally, collection and analysis of Chesapeake Bay menhaden will provide increased confidence in these literature-based estimates of PCBs.
annual PCB removal.

**WE151 New insights into biological sulfonamide degradation**

B. Ricken, University of Applied Sciences Northwestern Switzerland / Institute for Ecopreneurship; O. Fellmann, University of Applied Sciences and Arts Northwestern Switzerland

Although sulfonamides are among the most frequently administered antibiotics, there is a great interest in environmental fate (Hruska and Franek, 2012). Despite differences regarding abiotic degradation processes, all sulfonamide antibiotics have in common a poor biodegradability. Once released into the environment, even small concentrations are considered to enhance the propagation of sulfonamide antibiotic resistance genes. Just recently, several groups have published their successful isolation of bacteria being capable of partially mineralizing different sulfonamides. Overall, the strains are originating from highly diverse bacterial genera. *Microbacterium* sp. strain BR1, representing one of them, was isolated by our group. To the best of our knowledge, this is the only isolate for which the initial degradation step was described and identified as an ipso-substitution (Ricken et al., 2013). The identification of the same heterocyclic moiety as main metabolite in different studies leads to the assumption that the underlying degradation pathway among the different bacteria is similar. Furthermore, it was shown that the heterocyclic moiety itself does not possess antimicrobial activities, as the toxiphore group is represented by the sulfanilamide moiety (Majewsky et al., 2014). Different research groups could already proof the ability of bacterial isolates to grow on sulfonamides as the sole carbon source (Jiang et al., 2012; Reis et al., 2014; Ricken et al., 2013). Since the sulfanilamide moiety has to be mineralized, toxic intermediates might be formed, which have been investigated in this study. By degradation experiments of different possible intermediate compounds, and by the identification of formed metabolites by GC-MS analysis, we were able to postulate the downstream pathway during biological degradation of sulfamethoxazole with three novel identified intermediate compounds. The pathway proposed here is directly linked to the previously identified ipso-hydroxylation, representing the initial sulfonamide attack, and thus allowing us to present the whole sulfonamide degradation pathway just before the first ring cleavage product. Furthermore, we could show that even though possible toxic intermediates are formed, *Microbacterium* sp. strain BR1 seems to be able to transform them fast enough, circumventing their accumulation resulting in a toxification of the bacterial cell.

**WE152 Bioaccumulation of organic micropollutants in the cyanobacterium Microcystis aeruginosa**

M.A. Strays, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Chemistry; F. Pomati, Eawag Swiss Federal Institute of Aquatic Science and Technology / Aquatic Ecology; J. Hollender, Eawag / Environmental Chemistry

*Microcystis aeruginosa* is a cyanobacterial species prevalent in freshwater phytoplankton and among the most important bloom-forming species in eutrophic lakes. Using liquid chromatography - high-resolution mass spectrometry, we identified bioaccumulation of organic micropollutants of different substance classes in *Microcystis aeruginosa* in laboratory experiments. A set of strobilurin and conazole fungicides was studied. Strobilurin fungicides are used in agriculture, while conazole fungicides have uses in both agriculture and human medicine. For comparison and to further investigate the relationship between log Kow and bioaccumulation, a laboratory experiment was performed to estimate the effect of pure soil, pure ash and a mix of both on the cell density. Chlorophyll a content and maximum quantum yield of the microalgae *Raphidiopsis subcapitata*. For each treatment dilutions of 50% and 25% (diluted with MBL medium) were tested as well as a filtered 100% solution. Results showed that cell density and chlorophyll a content were negatively affected by only the highest concentrations of the mixed treatment and by the 50% diluted pure ash treatment, if compared to the control treatment. This observation was probably caused by ash toxicity rather than by light limitation, since cell density and chlorophyll a content of the soil treatments were not significantly affected. The maximum quantum yield was not influenced, except by all filtered treatments. Remarkably, the maximum quantum yield was significantly higher in filtrated treatments compared with control and other treatments. It was also shown that the 100% filtrated treatments lead to a significant decrease in cell viability, which primary production sustains aquatic life. Copper is an essential metal involved in the oxygen transport, while Cd has no apparent biological functions, although in some limiting conditions it can substitute Zn. However, Cu and Cd can be co-accumulated and mobilized to different compartments under stress conditions, although the dose may determine its real behaviour. The present work analyzes the impact of environmentally relevant concentrations of Cu and Cd on the growth, viability, cell size and photosynthetic activity of the tropical freshwater phytoplankton *Chlororobium braunii*. P. Echeveste, J. Carmona Silva, Universidade Federal de São Carlos / Department of Botany; C. J. Galbán, Sustainability Research Centre / Department of Ecology and Environment

**WE155 Size matters: Effects of Cu and Cd on the growth, viability, cell volume and photosynthetic activity of the tropical freshwater phytoplankton *Chlororobium braunii***

P. Echeveste, J. Carmona Silva, Universidade Federal de São Carlos / Department of Botany; C. J. Galbán, Sustainability Research Centre / Department of Ecology and Environment

Copper and Cd are two of the most ubiquitous trace metals in nature, including freshwater ecosytems. In these environments, their cycling is influenced by phytoplankton, which primary production sustains aquatic life. Copper is an essential metal involved in the oxygen transport, while Cd has no apparent biological functions, although in some limiting conditions it can substitute Zn. However, Cu and Cd can be co-accumulated and mobilized to different compartments under stress conditions, although the dose may determine its real behaviour. The present work analyzes the impact of environmentally relevant concentrations of Cu and Cd on the growth, viability, cell size and photosynthetic activity of the tropical freshwater phytoplankton *Chlororobium braunii*. Growth was significantly impaired by Cu and Cd, with EC50 occurring at 33.6 and 1.6 μM, respectively. At concentrations where no significant differences in cell abundances were observed, cell death was already induced, as observed by the increase in the percentage of dead cells, with EC50 occurring at 17.2 μM Cu and 1.1 μM Cd. Regarding the oxygen-evolving complex (OEC) of photosystem II (PSII), Cu had no significant effects on PSII maximum yield (ΦPSII) and the operational quantum yield (ΦPSII), while Cd contamination status of organochlorine pesticides (OCPs) in Korean coastal waters. OCPs (4 HCHs, HCB, heptachlor and its epoxide, 5 chlordanes, and 6 DDTs) were analyzed in eel samples. This is the first study to investigate residue levels and accumulation patterns of OCPs in eels from Korean coastal waters. Among OCPs, DDTs were the most frequently detected and showed the highest concentration. The next greatest concentrations were HCB and chlordanes. DDTs ranged from 2.73 to 144.8 ng/g wet wt, while the concentration of the total DDTs in eels was the main compound in DDTs, and its proportions accounted for 70%. HCB ranged from 0.14 to 1.26 ng/g wet wt and chlordanes ranged from less than detection limits to 1.12 ng/g wet wt. *trans*-nonachlor was the most abundant in chlordanes. There were significant differences in OCPs concentrations among sampling areas. Higher concentrations of DDTs, chlordanes, and HCB were found along industrialized and urbanized coasts. The mean concentration of DDT was the highest, and the ratio of (DDE+DDT)/DDTs was the lowest in the Yellow Sea. It suggests that this area has been input and polluted by fresh DDTs.

**WE154 Off-site effects of wildfires: Assessment of the effects of ash and soil on a freshwater microalgae**

N. Abrantes, University of Aveiro / CESAMDAO; A. Kieskamp, University of Aveiro / CESAM Department of Environment; V. Silva, Universidade de Aveiro / Department of Environment and Planning and the Centre for Environmental and Marine Studies CESAM; F. Gonçalves, S. Frankenbach, J. Serôdio, University of Aveiro / CESAM Department of Biology; J. Keizer; Department of Environment Center for Environmental and Marine Studies CESAM University of Aveiro

Wildfires are a common phenomenon in Portugal, with an average of 110,000 hectare burned every year. Wildfires physically degrade land, promoting runoff and erosion and consequently washout of ashes. This leads to an increase of suspended solids in aquatic ecosystems which may decline the aquatic habitat quality by limiting habitat availability and light penetration. In addition, ashes can contain metals and PAHs (Polycyclic Aromatic Hydrocarbons), which are known as toxic compounds inhibiting growth, development and reproduction of aquatic organisms like green microalgae. To analyze the off-site effects of ash and soil on primary production, a laboratory experiment was performed to estimate the effect of pure soil, pure ash and a mix of both on the cell density, chlorophyll a content and maximum quantum yield of the microalgae *Raphidiopsis subcapitata*. For each treatment dilutions of 50% and 25% (diluted with MBL medium) were tested as well as a filtered 100% solution. Results showed that cell density and chlorophyll a content were negatively affected by only the highest concentrations of the mixed treatment and by the 50% diluted pure ash treatment, if compared to the control treatment. This observation was probably caused by ash toxicity rather than by light limitation, since cell density and chlorophyll a content of the soil treatments were not significantly affected. The maximum quantum yield was not influenced, except by all filtrated treatments. Remarkably, the maximum quantum yield was significantly higher in filtrated treatments compared with control and other treatments. It was also shown that the 100% filtrated treatments lead to a significant decrease in cell viability, which primary production sustains aquatic life. Copper is an essential metal involved in the oxygen transport, while Cd has no apparent biological functions, although in some limiting conditions it can substitute Zn. However, Cu and Cd can be co-accumulated and mobilized to different compartments under stress conditions, although the dose may determine its real behaviour. The present work analyzes the impact of environmentally relevant concentrations of Cu and Cd on the growth, viability, cell volume and photosynthetic activity of the tropical freshwater phytoplankton *Chlororobium braunii*. P. Echeveste, J. Carmona Silva, Universidade Federal de São Carlos / Department of Botany; C. J. Galbán, Sustainability Research Centre / Department of Ecology and Environment

**Organochlorine pesticides in eels, Conger myriaster, from Korean coastal waters**

M. Choi, I. Lee, D. Hwang, R. Jung, National Fisheries Research and Development Institute

As eels have a high fat content, lipophilic contaminant concentrations can be relatively high. Therefore, monitoring of contaminants in eel seemed very informative from a human health point of view, as this type of fish could serve as a worst-case scenario. In particular, eel has some remarkable properties that make it a very attractive biomonitor. Eels (*Conger myriaster, 30-35 cm length*) were collected from Korean coastal waters in April-August, 2014, in order to investigated
significantly impacted $\Phi_{Q_n}$ with EC50 occurring at 18.4 $\mu$M. The photochemical fluorescence quenching ($Q_p$) and non-photochemical quenching ($Q_n$) responded differently to Cu and Cd. A significant decrease of $Q_p$ was observed after Cd addition, but not for Cu, which showed almost no variation. In contrast, Qn values significantly increased after both metal additions, gradually in the case of Cd and after addition of high concentrations in the case of Cu. Non-photochemical quenching due to heat dissipation (NPQ) significantly increased in response to both metals, accumulate in plants and aquatic organisms. The present study was undertaken to investigate the presence of POPs in some dams of Mexico, through its determination in muscle of fish (Oreochromis sp.). POPs extraction was performed using ultrasonic probe assisted extraction, detection and quantification by gas chromatography-mass spectrometry. In the fishes of the muscle the presence of some organochlorine pesticides (Aldrin, HCH, DDT, DDE) and polychlorinated biphenyls (PCBs) was recorded. Acknowledgements: CONACYT-SEMARNAT-2008-1-108334

WE157 Effects of climate change-induced physical disturbance on sediment deposited pollutants and their interactions with bacteria in the Northern Baltic Sea. C. Gallampois, Umea University / Chemistry: P. Haglund, Department of Chemistry; G. Rowe, Department of Ecology and Environmental Science Umea University

Christine Gallampois1, Peter Haglund2, Owen Rowe3,1 1Chemistry Department, Umea University, Umea, Sweden; 2Department of Ecology and Environmental Science, Umea University and totally inhbitited in the oligotrophic medium. In addition, the highest accumulation of arsenic was found in the plant’s roots. After these in vitro experiments, complementary experiments in mesocosms are in progress. Further, in situ experiments including watermiiolof reimplantation should help to refine these results. Keywords: Myriophyllum alterniflorum, elements traces métalliques, spéciation, biomarqueurs.

WE160 Effect of an herbicide on the growth rate of freshwater primary producers A.M. Gonçalves, Biology; D.V. Barroso, University of Coimbra / Department of Life Sciences; T. Vidal; J.C. Marques, IMAR-Institute of Marine Research, Department of Life Sciences, University of Coimbra / Department of Life Sciences; F. Goncalves, University of Aveiro CESAM / Department of Biology. Herbicides are widely used to control pests and diseases in crop production. Among these agrochemicals, herbicides are commonly applied on crops to control adventive infestations. Once in the aquatic system, these chemicals may disturb aquatic organisms leading to underperformance of the most sensitive species, causing ecosystem unbalance. Aquatic plant toxicity tests are frequently conducted to assess environmental risks and to determine the potential impact of contaminants on primary producers. Among aquatic organisms, Chlorella vulgaris, Raphidocelis subcapitata (green freshwater microalga) and Lemma minor (aquatic vascular plant) are commonly used in phytotoxicity tests because of their small size, their high reproductive rate and the facility with which organisms handle facilities. The replication of C. vulgaris and L. minor was assessed. Watermilfoil was more sensitive to Cd than Cu, with growth and viability being more sensitive than maximal and effective photosynthetic efficiencies, although non-photochemical quenchings were severely damaged by both metals.
herbicide, the food quality and availability to higher trophic levels may be threatened.

WE161
Assessing the soil microbial toxicity of trifloxystrobin and its main soil metabolite using advanced biochemical and molecular tools
N.A. Stuegi, C. Kropscott, Dow Corning Corporation / Institute of Agricultural and Environmental Chemistry; E. Puglisi, Universita Cattolica del Sacro Cuore / Microbiology; S. Vasileiadis, University of South Australia / Centre for Environmental Risk Assessment and Remediation; C. Oplos, Aristotle University of Thessaloniki / Pesticide Science Laboratory; F. Fornaser, Centro lo Studio delle Relazioni tra Pianta e Suolo / Consiglio per la Ricerca in Agricoltura; S. Sachdeva, University of Reading / Department of Agriculture, Policy and Environment; K. Karpoyzas, University of Thessaly / Department of Biochemistry and Biotechnology; M. Trevisan, Universita Cattolica del Sacro Cuore / Institute of Agricultural and Environmental Chemistry

Trifloxystrobin, methyl-(E)-methoxyimino-(E)-a-[1-(a, a-trifluoro-m-tolyl) ethylidenaminoiminio]-o-toly acetate, is a strobilurin fungicide, a new class of substance that is included in the Quinone outside inhibitors (QoIs) fungicide groups. Trifloxystrobin is used for control of various diseases of fruits and vegetables. The major metabolite of trifloxystrobin in soil is CGA 312113 (E,E)- Methoxyimino-2-[1-(3-trifluoro methylphenyl)-ethylidenaminooxymethyl]-phenyl-acetic acid. This acid metabolite is included in the residue definition for risk assessment in plant commodities. Little is known about the impact of trifloxystrobin or its main soil CGA 311113 metabolite on the structure and function of the soil microbial community. For addressing associated knowledge gaps concerning the potential toxicity of trifloxystrobin and CGA 311113 on the soil microbial community we studied the effects of four dose levels (namely x0, x1, x10 and x100 the recommended dose) of trifloxystrobin in soil in a microcosm approach. Key soil microbial enzymatic activities and total bacterial diversity shifts using Illumina sequencing of the 16S rRNA gene amplicons were screened and compared with the dissipation data of trifloxystrobin and formation/degradation of CGA 311113. Dissipation assessment revealed that the half-life of trifloxystrobin in soil was less than 5 days, at all application rates, and the highest formation rate of the metabolite was observed in the first 15 days. Trifloxystrobin was correlated positively with the potential nitrification activity (PNT). No effect of trifloxystrobin on the measured extracellular enzyme activities (acid-phosphatase, beta-glucosidase, leucine-aminopeptidase, phosphomonoesterase, phosphodiesterase, chitinase) could be extrapolated according to the performed statistical analysis. However, distance based redundancy analysis (db-RDA) showed a time effect on enzymatic activities within the different trifloxystrobin application rates. Bacterial diversity analysis is on the way and the complete dataset will be presented during the conference.

Innovations in environmental analytical chemistry: the quest for pollutants using target and non-target approaches (P)

WE162
Determination of selected steroid hormones in some farmland pond, surface water and wastewater channel Cape Town environment using HPLC
O.S. Olutunji, Cape Peninsula University of Technology / Chemistry; B.O. Opeolu, Cape Peninsula University of Technology / Faculty of Applied Sciences; O.S. Fatoki, B.I. XMDA, Cape Peninsula University of Technology / Chemistry

Steroidal hormones are widely used in livestock farming such as poultry, cattle farming, and in veterinary and human medicine. Over two-third of the drugs are not used, hence it is excreted in urine and faecal wastes in the form they are administered, or as metabolites with strong resemblance to the parent compound. Consequently, they are found in different environmental matrices such as water. To protect the environment, exposure to residues of steroid hormones was reported to disrupt the endocrine systems even at low concentration. This study evaluated the concentration levels of selected steroid hormones in selected water streams, including farmland pond, surface water and wastewater treatment plant effluent in Cape Town. Water sample were collected in nicotinic acid pre-washed amber coloured bottles from columns of some water receiving pond within some animal rearing farms located in Athlone, Glenwood and the area around the Bell Downs in Cape Town environment. The samples were prepared using standard methods. Steroid hormones were recovered from the samples in 50% methanol using solid phase extraction (HILB), cleaned and concentrated to 2ml under nitrogen stream for analysis. The hormones were separated, detected and quantified for the estriol, bisphenol-A and estrone using UHPLC-DAD. The results showed that the concentrations of estriol and estrone in all the water samples were variable, while Bisphenol-A did not occur at detectable levels. The concentration of estriol and estrone ranged between 0.0126 – 0.0131 μg/L (0.0128±0.0021 μg/L), 0.0154 – 0.0159 μg/L (0.0157±0.0021 μg/L), respectively. Estril in water may be the result of run off from the cattle on the farm in Blue Downs, with the levels detected in pond 1 higher than in pond 2 which is in contact with a stream. Contrary to expectation, the Athlone waste water treatment plant had higher levels of estradiol compared to the farmland pond. The water treatment procedure may have exacerbated their levels. The concentration levels of steroid hormones in all the water sample tested were observed to be slightly below the 0.02 mg/L of estrogen suggested by the SADC Ministry of Health, except for estriol.

WE163
A study of analyte recovery as a function of analyte concentration and sample storage time using headspace solid-phase micro-extraction (HS SPME) coupled with GC-ECQ
E.M. Donnivan, Whitman College / Chemistry

Headspace Solid-Phase Microextraction (HS SPME) has been shown to recover pesticides and PCBs from aqueous samples at the expected ultra-trace levels (parts per quadrillion) of alpine lakes. Several variables of the HS SPME technique have been adequately evaluated including water temperature and length of fiber exposure, but surprisingly analyte recovery as a function of analyte concentration and sample storage time has not been systematically evaluated. In this study, we have evaluated the recovery of the chlorinated hydrocarbons 4,4’-dichlorophenyltrichloroethane (p,p’-DDE), 2,4’-dichlorophenyltrichloroethane (o,p’-DDE), 4,4’- dichlorodiphenylchloroethane (DDE), 2,4’-dichlorodiphenylchloroethene (o,p’-DDE), chlorothalonil-dimethyl gamma-hexachlorohexane (lindane), heptachlor, and trans-nonachlor. Evaluated concentrations ranged from parts per quadrillion to parts per billion in spiked water samples and data indicate that recoveries in the ppq range can be as low as ten percent. In light of the low SPME recoveries in the ppq range, several previously reported data sets for chlorinated hydrophobic analytes measured in alpine lakes need to be re-evaluated. The HS SPME technique was further used to determine the concentration of chlorinated pesticides in several U.S. alpine lakes.

WE164
Automated Derivatization, SPE Cleanup and LC-MS/MS Determination of Glyphosate and Others Polar Pesticides
J. Stahl-Zeng, AB SCIEX; D. McMillan, Sciei; A. Sage, ABSCIEX / marketing

Glyphosate is a common broad-spectrum systemic herbicide used widely to kill weeds especially annual broadleaf weeds and grasses known to compete with crops. There is an interest in the reliable and sensitive quantitation and identification of glyphosate residues, its metabolite AMPA, and the related glufosinate in food and water. Commonly large volume injection into ion chromatography or LC systems based on HILIC followed by sensitivity MS/MS detection is used for analysis. However, interference can influence results in complex samples since the method does not use any cleanup. Derivatization techniques can be used successfully. The method presented here uses derivatization with FMOC-CI followed by automated SPE cleanup using a Gerstel front-end and detection using LC-MS/MS with an AB SCIEX QTRAP 4500 system. Limits of quantitation in food were found below the target 100 μg/kg allowing dilution to minimize potential ion suppression. In drinking water samples a method development was performed for the variety of over three orders of magnitude with r > 0.999 was observed with excellent reproducibility because of the complete automation of the sample handling procedure.

WE165
Challenges in Analytical Method Development and Validation for Trace-Level Dimethyldisiloxane in Water
S. Xu, Dow Corning Corporation / Health and Environmental Sciences

Dimethyldisiloxane (DMSD) is not a major ingredient of any commercial products. However, it can be produced in soil and water by hydrolysis of methylsiloxanes in the environment. Due to widespread application of methylsiloxanes in commercial products and its demonstrated degradation in soil and water, the occurrence of DMSD in water. The identified challenges include the difficulty to isolate DMSD from water, high background contaminations and poor chromatography of the extracted DMSD. Both pre-column derivatization and direct analysis methods were evaluated for overcoming the above challenges to achieve adequate sensitivity and accuracy. As the result of those evaluations, a method consisting of solid-phase extraction, reaction of DMSD with 1,2-Dichloroethane (DCE) in water, removal and direct GC/MS analysis has been developed and validated for natural water at a method detection limit for DMSD close to 10 μg L⁻¹.

WE166
Analytical Method for Trace-Level Trimethylsilanol in Water
S. Xu, Dow Corning Corporation / Health and Environmental Sciences; B. Kropscott, Dow Corning Corporation

Organosiliconc materials such as trimethylsilanol and dimethyldisiloxane are two reactive compounds and ultimate hydrolysis products of a group of high-production-volume per-methylated organosilicones, in the environment. The distribution of these organosilicon compounds in various environmental media is, therefore, very important for a complete understanding of the fate and effects of siloxanes. Although the environmental information on siloxanes is exponentially increased in recent years, the data on environmental occurrence of siloxanes are scarce. This is largely due to the lack of sensitive methods for accurate analysis of low level silanols in the environmental
media. In the present studies, we have developed highly sensitive methods for accurate analysis of trace-level silanols in water. This presentation will focus on the development and validation of a method capable of analyzing sub-pb level TMS in natural water based on solid-phase extraction (SPE) coupled with gas chromatography/mass spectrometry (GC/MS).

**WE167**

**Study of Chemcatcher® kinetic uptake for herbicide: batch experiment and flow laboratory calibration**

I. Imtiaz, Ecole des Mines d’Alsace / Laboratoire LGEE; A. togola, BRGM / Laboratory Division; R. Greenwood, University of Portsmouth / School of Biological Sciences; C. González, Ecole des Mines d’Alsace / LGEE

This paper presents the kinetic uptake of selected herbicides by a commercial sampler named Chemcatcher® (C18 as receiving phase and PES membrane) has been determined by the two methods. Based on a kinetic approach two methods of calculating Rs are proposed: one based on batch experiments, and the other on through flow laboratory calibration. The results of the batch experiment are in good agreement to those of flow laboratory calibration and the former approach has the advantage of simplifying the calibration procedure for determining Rs. However, since hydrodynamic conditions can vary widely in the field, caution is needed when using laboratory based calibration data in deployments.

**WE168**

Zwitterionic based online solid phase extraction liquid chromatography tandem mass spectrometry for rapid environmental trace analysis of the very polar and hydrophilic antiviral drug zanamivir

H.S. Söderström, Umea University; G. Fedorova, University of South Bohemia in Ceske Budejovice / Faculty of Agriculture; C.A. Galinaro, Universidade de Sao Paulo / Chemistry; F.M. Pereira, University of São Paulo / Chemical Engineering.

The zwitterionic-based online SPE/LC method showed acceptable linearities (r² of 95/5 v/v ACN/aqueous sample and then 1% FA). Furthermore, the developed method showed acceptable linearity (r² > 0.994), filtration recovery (≥ 91%), and repeatabilities (RSD ≤ 16%), and LOQs values which were acceptable and environmentally relevant (≤ 20 ng L⁻¹). The evaluation also showed that MililiQ water, surface water and sewage effluent can be stored for 20 days in +4 and -20 °C (losses ≤ 12%) while sewage influent should be stored at -20 °C to avoid significant ZA losses.

**WE169**

Validation of a method for the determination of chlorothalonil in seawater.

G. Zanuto, University of São Paulo / USP; L.R. Diniz, T.M. Oliveira, São Paulo State University Institute of Chemistry; E.M. Vieira, Sao Paulo University / Departamento de Química e Física Molecular

The formulation of marine antifouling coatings can include, inorganic, organic or organometallic substances, which have the function to inhibit the attachment of organisms (biofouling) that can compromise the mechanical structure, fuel consumption and reduce the useful lifetime of ship’s hulls. With the restrictive regulation of the TBT, new compounds have been incorporated along with copper the composition of paints. In Brazil there are few studies focusing on such compounds. These substances are used separately or in mixtures, in the latter case to enhance the biocide action of painting. Recent studies report that increased use of antifouling bottom paints may cause certain environmental problems.

Chlorothalonil (2,4,5,6-tetrachlorothiophosphorilniltrile) is a fungicide belonging to the group of isothianilnitrils with a molecular weight of 265.9 g mol⁻¹, has a logKow of 2.88 and a BCF of 264, which indicates that is not considered bioaccumulative, however degrades to a persistent metabolite ecotoxic. The half-life for its degradation in the environment is 150 hours, while the half-life for the metabolite is greater than 30 days is therefore considered persistent as it forms persistent toxic degradation products. Chlorothalonil starting been used also as booster antifouling paints third generation and despite other uses as a wood protectant, pesticide, acaricide, and to control mold, mildew, bacteria and algae, the presence of chlorothalonil in samples of seawater may indicate its source is associated with for example the use of biocide. Target compound was isolated by liquid-liquid extraction (LLE) and then analyzed by by HPLC-DAD using analytical column C18 Nucleosil (150 x 4.6mm, 5 μm endcapped), and gradient mobile phase containing acetonitrile and phosphoric acid 0.1% 70:30 at 1.0 mL min⁻¹. Detection and quantification were made at a wavelength of 231 nm. Preliminary results were the establishment and validation of the analytical conditions for quantification of chlorothalonil, for determination of sensitivity and linearity of the method have been established parameters of the calibration curve for concentrations between 0.1 and 4 μg L⁻¹. The correlation coefficients (R) obtained, was 0.99 and limit of detection and limit of quantification 1.0 and 2.0 μg L⁻¹ respectively Keyworks: Antifouling paints, chlorothalonil, LLE.

**WE170**

The spatial and temporal pesticide and metal contamination in Dilek National Park, Turkey

C. Turgut, ADU; L. Atatanir, Adnan Menderes University; M. Yalçın, Adnan Menderes University / Faculty of Agriculture; T. Ciftug, University of Akdeniz / Department of Civil Engineering; A. Gavcar, Adnan Menderes University / School of Biological Sciences; I. Imtiaz, Ecole des Mines d’Alès / Laboratoire LGEI; a. togo

The pollution of water by pesticides is an issue of environmental concern, owing to the widespread use of pesticides in agriculture activities leads to the contamination of surface and ground waters caused by drift, runoff, drainage, and leaching of soils. The pollution of water by pesticides is an issue of environmental concern, owing to the increasing number of pesticides detected in water and to the establishment of directives such as Directive 2000/60/EC which defined a framework for the protection of waters bodies. A number of pesticides belonging to the classes of phenylureas, triazines and chloroacetanilides, are mentioned as priority substances. Passive sampling techniques, which rely on the diffusion of pollutants from the sampled medium to a receiving phase, have gained in popularity because they can provide time-weighted average (TWA) concentrations of pollutants when used in an integrative mode. Since these devices accumulate pollutants over a period of time, they can, depending on the compound-dependent sampling rate, provide a priori concentration of the analyte and also for pesticides, a dose is delivered to a constant analytic concentration in water. This work aims to compare the sampling rate Rs obtained through flow pilot calibration and batch experiments for 20 polar herbicides. The uptake of herbicides by the Chemcatcher® (C18 as receiving phase and PES membrane) has been determined by the both methods. Based on a kinetic approach two methods of calculating Rs are proposed: one based on batch experiments, and the other on through flow laboratory calibration. The results of the batch experiment are in good agreement to those of flow laboratory calibration and the former approach has the advantage of simplifying the calibration procedure for determining Rs. However, since hydrodynamic conditions can vary widely in the field, caution is needed when using laboratory based calibration data in deployments.

**WE171**

**SEASONAL VARIATIONS IN CONCENTRATION OF PARABENS ON MOGI RIVER IN BRAZIL**

C.A. Galinaro, Universidade de Sao Paulo / Chemistry; F.M. Pereira, University of São Paulo / USP / engineering of materials; E.M. Vieira, Sao Paulo University / Departamento de Química e Física Molecular

Seasonal variations in concentration of parabens on Mogi River in Brazil

Carlos A. Galinaro, Fabiana M. Pereira and Eny Maria Vieira* IQSC - Universidade de São Paulo - Brazil. *e-mail eny@usp.br. Keywords: Parabens, surface water, and DLLME-HPLC-DAD. Parabens are preservatives extensively used to prevent microbial growth in many common care products. Most parabens are frequently found in raw water at concentrations reaching from ng L⁻¹ to mg L⁻¹, and their levels depend mainly on the extent of water dilution resulting from rainfall. Discharge of treated wastewater effluent into the river was found to...
be the main cause of water contamination with parabens. Dispersive liquid-liquid microextraction (DLLME) is an analytical technique, based on the dispersion of extraction solvent assisted with a disperser solvent within an aqueous solution that generates a very high contact area between the aqueous phase and the extraction solvent. The present study reports the application of a DLLME-HPLC-DAD for the simultaneous separation and determination of four paraben preservatives (methyl-, ethyl-, propyl-, and n-buty1-paraben) in surface water samples. The extraction of samples was performed using a 50 uL sample loop with autosampler; gradient generated in situ by Thermo Scientific Sionex HPLC. Analytical conditions: Column Thermo Scientific IonPac AS19, 1.5 μm, 75 mm x 4 mm; conductivity detector; gradient elution with 30% of 0.005 M LiNO₃/70% of 0.5 M NaNO₃ at a flow rate of 1 mL/min, followed by 60% of 0.005 M LiNO₃/40% of 0.5 M NaNO₃ at the same flow rate; post column derivation to obtain compound with a chromophore group to be detectable. The performance of the MIP is evaluated by the comparison between the classical method for the quantification of glyphosate and AMPA in water samples (on line SPE/UPLC/MS/MS after derivatization) with those obtained after MIP-SPE clean up method. Results have shown that the MIP at a SPE clean up method allows the catchment both molecules in real water matrices which present different physico-chemical properties. The influence of metals concentration in samples on the recovery rates was also studied as metals are likely present in severa1 environmental samples collected from different geographies and from different type of water, including drinking water, creeks, rivers, lakes, sea etc. All samples were analyzed by direct injection Liquid Chromatography coupled to tandem Mass Spectrometry (LC/MS/MS). Two analytical methods were used. A method which enables the quantification of PPCP at low ppt levels using Multiple Reaction Monitoring (MRM) and their identification using full scan MS/MS with mass spectral library searching using a hybrid triple quadrupole linear ion trap LC-MS/MS system (QTRAP® 6500). Analytical performance characterization of 18 compounds was performed. Accurate mass LC/MS/MS system the (TripleTOF™ 5600+) was used to further explore collected samples for unexpected analytes. Data processing turned out to be the bottleneck of the general unknown screening methodology. New and advanced data processing tools where used to automatically identify unexpected and unknown pollutants. Key words: unknown screening, PPCP in water, quantification, identification

WE173 New molecularly imprinted polymers (MIP) used as SPE clean up method and as a passive sampler receiving phase for the catchment of glyphosate and AMPA in water C. Berho, BRGM / Laboratory Division; B. Claude, Université Orléans / ICoA UMR, L. Amalric, BRGC, a. togola, BRGM / Laboratory Division; E. Grelet, BRGM; S. Bayoudh, POLYINTELL; K. Puzio, P. Morin, Université Orléans / ICoA UMR Glyphosate is a broad-spectrum systemic herbicide which is widely used in the world. As very polar molecules, glyphosate and its main degradation product AMPA are difficult to extract with organic solvents and common solid phase extraction (SPE) sorbents. A new SPE sorbent based on Molecularly Imprinted Polymers (MIP) was thus developed for Glyphosate and AMPA. The performance of the MIP is evaluated by the comparison between the classical method for the quantification of glyphosate and AMPA in water samples (on line SPE/UPLC/MS/MS after derivatization) with those obtained after MIP-SPE clean up method. Results have shown that the MIP at a SPE clean up method allows the catchment both molecules in real water matrices which present different physico-chemical properties. The influence of metals concentration in samples on the recovery rates was also studied as metals are likely to form complexes with glyphosate in natural waters. This sorbent was also tested as an integrative passive sampler (POCS) receiving phase since the classical version of POCS with OASIS HLB receiving phase cannot catch these molecules of interest. The calibration of POCS-MIP in laboratory conditions has led to laboratory sampling rates estimation for AMPA and glyphosate which are necessary for future field application of POCS-MIP. We acknowledge financial support from French National Research Agency (ANR ECOTECH 2011 ORIGAMI project 11 ECOt 003) Key words: molecular imprinted polymers, passive sampling, POCS, AMPA, glyphosate

M. LEVI, SHIMADZU France / Applications; S. MOREAU, Shimadzu Europa GmbH
As monitoring of drug residue in drinking and surface water is becoming of major concern in western countries, the environmental laboratories must face the increase in analysis request as well as the increase in the number of compound to be screened. In parallel, as long as no consensus has been found on regulatory reporting levels, the assay sensitivity must be very high to not miss any compound. Therefore, laboratories need a simple, rapid, reliable, sensitive and cost effective method to screen and accurately quantitate drug residues in a variety of water samples. A method was then set up to fulfill these requirements. On line SPE is a useful tool to enable large sample volume injections. But due to the chemical diversity of target compounds, sample clean up can not be pushed too far to not lose polar compounds. Then matrix effect or ion suppress are can be problematic for accurate quantification. Then, a very sensitive Mass Spectrometer as well as very efficient chromatography were necessary to compensate the relatively low sample volume injection. The method presented is able to screen and accurately quantitate around 150 drugs or metabolites in about 10 minutes (including sample preparation). Compounds were from diverse pharmaceutical classes like NSAIDs, steroids, antibiotics, psychotropics or antidepressants. Ultra fast polarity switching was found to be essential to ensure data quality. Validation results are presented, including accuracy in several matrices, as well as a comparison with a direct injection method.

WE175 Determination of an On-line SPE LC/MS/MS method for the analysis of antibiotics in waste- and surface water A. Meiromjohann, Abu Akademi University / Laboratory of Organic chemistry; E. Krymzyn, Abu Akademi University / Labotory Of Organic Chemistry; L. Kronberg, Abu Akademi University
Antibiotics are one of the most important classes of pharmaceuticals having made a large number of previously often fatal bacterial infections easily curable. Because of their high production use and the increasing occurrence of resistance, the increased appearance of multi-drug resistant bacteria in recent years has caused concern. An expected hot spot for the formation and proliferation of resistance genes is wastewater treatment plants (WWTPs), where a continuous flow of sublethal concentrations of antibiotics and a large bacterial community come together. It has long been known that pharmaceuticals that have been consumed and subsequently excreted can be found in WWTPs where they are often partially removed in the activated sludge process and then released in the recipient environmental waters. Analysis of trace amounts of organic contaminants in environmental samples is generally an expensive and time consuming work. In this work we have developed a fast and cost efficient On-line SPE LC/MS/MS method for the determination of antibiotics and their metabolites. An on-line SPE LC/MS/MS method was used to screen the compounds, sample clean up can not be pushed too far to not lose polar compounds. Those in which the highest consumption of products containing preservatives (food, personal care products, pharmaceuticals hygiene, and others). Acknowledgment: This work was supported by CNPq (150540/2013-2).
by association to particulate matter and subsequent deposition in the sediment. The proposed analytical method is efficient for the determination of compounds of interest displayed good accuracy. Recoveries were between 70 and 120% for both compounds. The samples show different realities in the state, but herbicide and its degradation products residues were found in both agricultural municipalities as in São Luís, a more urbanized and industrial city.

WE177 The Use of Molecular Markers to Quantify Estrogens in Natural Waters by LC-MS/MS

J. Hendricks, J.J. Harwood, Tennessee Technological University / Chemistry

Molecules et Materiaux du Mans IMMM; G. Louarn, University of Nantes / Institut des Matériaux Jean Rouxel IMN

Wood has been used as an important material of human life for thousands of years. Wood can be applied widely in both everyday life and industry as tools, construction materials and fuels. However, for applications and for longer preservation, wood has to be chemically treated with pesticides or some molecules those are known to be toxic. Two major kinds of chemical pollutants are used: heavy metals (typically cadmium, lead, copper and others) and organophosphates such as polybrominated diphenyl ethers (PBDEs), pentachlorophenol (PCP) and creosote. Wood products, at the end of their using life, are dischargeable and could be recycled but the presence of these toxic molecules in waste wood hinders their recycling process. Besides, the chemical molecules present in wood can be washed by rain and end up in rivers and surface waters, which is of course a strong environmental concern. Therefore, rapid detection and classification of these molecules in wood items are necessary and are a potential demand from the industries involved in wood recycling. Indeed, in France, the recycling companies are still classifying waste wood relyingly mostly on their sources, without any analytical procedure. Three classes are considered: A for clean wood, B for wood treated with non-hazardous material and C for the wood treated with hazardous materials. Nowadays only classes A and B can be recycled, mainly for particleboard and combustion fuel, and only heavy metals contamination is monitored by X-Fluorescence. The detection of organic pollutants lacks of a really efficient technique. In this work, two techniques were tested to detect organic pollutants in wood items: i) Raman spectroscopy which is a fast, non-destructive and non-invasive fingerprint technique for detection of organic molecules that can be used in situ. Associated with chemometrics, Raman spectroscopy was used in this work to discriminate wood items by classes and monitor the presence of pollutants. ii) Surface Plasma Resonance imaging (SPRI), an innovative technique that can provide a sensitive and real-time measurement for molecular detection, was also used for non-target detection of organic pollutant in wood recycling leachate. The advantages of both techniques for a quick detection and quantification of organic pollutants in waste wood and leachate from wood industry will be presented.

WE180 New sensor for direct detection of pesticides in water by Raman spectroscopy coupled with enzymatic functionalized nanoparticles

A. El ALAMI, Institute of Materials and Molecules of Le Mans IMMM UMR CNRS; f. lagarde, Institute of molecules and materials of Le Mans / Institute of Materials and Molecules of Le Mans UMR CNRS; B. MIMOUNA, Faculté des Sciences Dhar Mehraz Fès / Laboratoire de Physique du Solide équipe polymères et nanomatériaux; P. Daniel, University of Le Mans / Institut des Molecules et Matériaux du Mans IMMM

Pesticides are worldwide used in agriculture and industries. Some of them are highly toxic and may perturb some vital functions in organisms. In this framework it is very important to dispose of tools able to detect very fast and with very high sensitivity these kinds of compounds. In this work an acetylcholinesterase (ACHE) biosensor was developed in the goal of a direct detection of pesticides in water. Our method is based on Surface Enhanced Raman Scattering (SERS) phenomenon which is based on the increasing of a Raman signal when an analyte is adsorbed on the surface of a silver or gold substrate in close contact with a multilayer metal film. The detection of pesticides is based on the enzymatic activity of the enzyme and to a different Raman spectrum. In this study, paraoxon and carbaryl were used as model pesticides and the first results showed that this biosensor gave highly sensitive and very fast responses. This biosensor can be used for a non-specific and on-line detection of enzyme inhibitors.

WE181 Analysing Personal Care Products in biota by liquid chromatography - atmospheric pressure photoionisation - mass spectrometry

D. Molina Delgado, Environmental Chemistry; R. Muñoz-Condé, IDAEA-CSIC; S. Diaz-Cruz, IDAEA-CSIC / Environmental Chemistry; D. Barcelo, IQIAB-CSIC / Institute for Environmental Assessment and Water Research

Personal care products (PCPs) is a generic term that describes a group of organic chemicals that are included in different products widely used in daily practices (such as toothpaste, shampoo, cosmetics and even in food), being used in considerable quantities. After use, they may be absorbed by the body and excreted or washed after its application. PCPs and their metabolites enter the aquatic environment and reach WWTPs [1], where they are aplitically eliminated [2]. On recent times, the concern about the potential hazardous risk associated to them and their byproducts [3], has been on the rise. Traditionally, the analysis of PCPs have been carried out limiting the amount of compounds to a few per analysis. Fragrances have been analyse using gas chromatography (GC), whereas UV filters and parabens are detected and enhanced giving a specific SERS spectrum, reference spectra of a "healthy" enzyme. In presence of pesticide, the enzyme is inhibited leading to the absence of enzymatic reaction and to a different Raman spectrum. In this work an acetylcholinesterase (ACHE) biosensor was developed in the goal of a direct detection of pesticides in water. Our method is based on Surface Enhanced Raman Scattering (SERS) phenomenon which is based on the increasing of a Raman signal when an analyte is adsorbed on the surface of a silver or gold substrate in close contact with a multilayer metal film. The detection of pesticides is based on the enzymatic activity of the enzyme and to a different Raman spectrum. In this study, paraoxon and carbaryl were used as model pesticides and the first results showed that this biosensor gave highly sensitive and very fast responses. This biosensor can be used for a non-specific and on-line detection of enzyme inhibitors.

WE2182 Rapid detection and classification of organic pollutants by Raman spectroscopy and Surface Plasmon Resonance imaging: application to wood recycling industry

H.Q. Nguyen, Universite du Maine / Institut des Molecules et Matériaux du Mans IMMM; f. lagarde, Institute of molecules and materials of Le Mans / Institute of Materials and Molecules of Le Mans IMMM UMR CNRS; P. Daniel, University of Le Mans / Institut des Molecules et Matériaux du Mans IMMM; G. Louarn, University of Nantes / Institut des Matériaux Jean Rouxel IMN

Wood can be applied widely in both everyday life and industry as tools, construction materials and fuels. However, for applications and for longer preservation, wood has to be chemically treated with pesticides or some molecules those are known to be toxic. Two major kinds of chemical pollutants are used: heavy metals (typically cadmium, lead, copper and others) and organophosphates such as polybrominated diphenyl ethers (PBDEs), pentachlorophenol (PCP) and creosote. Wood products, at the end of their using life, are dischargeable and could be recycled but the presence of these toxic molecules in waste wood hinders their recycling process. Besides, the chemical molecules present in wood can be washed by rain and end up in rivers and surface waters, which is of course a strong environmental concern. Therefore, rapid detection and classification of these molecules in wood items are necessary and are a potential demand from the industries involved in wood recycling. Indeed, in France, the recycling companies are still classifying waste wood relyingly mostly on their sources, without any analytical procedure. Three classes are considered: A for clean wood, B for wood treated with non-hazardous material and C for the wood treated with hazardous materials. Nowadays only classes A and B can be recycled, mainly for particleboard and combustion fuel, and only heavy metals contamination is monitored by X-Fluorescence. The detection of organic pollutants lacks of a really efficient technique. In this work, two techniques were tested to detect organic pollutants in wood items: i) Raman spectroscopy which is a fast, non-destructive and non-invasive fingerprint technique for detection of organic molecules that can be used in situ. Associated with chemometrics, Raman spectroscopy was used in this work to discriminate wood items by classes and monitor the presence of pollutants. ii) Surface Plasma Resonance imaging (SPRI), an innovative technique that can provide a sensitive and real-time measurement for molecular detection, was also used for non-target detection of organic pollutant in wood recycling leachate. The advantages of both techniques for a quick detection and quantification of organic pollutants in waste wood and leachate from wood industry will be presented.
targeting important functional groups of bacteria were used. Preliminary results of the RNA extractions from the coarse sediment (low OC) showed 4 times less concentration of RNA per gram of sediment than the fine sediment (high OC), suggesting less microbial activity, however this is not necessarily traduced in less biodegradability. The DGGE analysis will indicate shifting and diversity of the microbial population which might explain the biodegradation results better than a total quantitation of the active microbial populations. This work is not finished but it can be concluded already that the vitality of microbial diversity is needed and further studies to evaluate in deep the differences observed.

WE184 PTR-ToF-MS as novel tool for real-time tracking nanopalladium(catalyzed transformation reactions of persistent organohalogen compounds}
E.C. Fischer, University Giessen / Institute of Soil Science and Soil Conservation; L. Bohm, Justus Liebig University Giessen / Institute of Soil Science and Soil Conservation Research Centre for BioSystems Land Use and Nutrition IFZ; M. Müller, University of Innsbruck / Institute for Ion Physics and Applied Physics; F. Schmidt, Institut for Environmental Research, M. Schlüter, Justus Liebig University Giessen / Institute of Applied Microbiology Research Centre for BioSysystems Land Use and Nutrition IFZ; F. Axt, Justus Liebig University Giessen / Institute of Soil Science and Soil Conservation Research Centre for BioSystems Land Use and Nutrition IFZ. A. Wisthaler, University of Innsbruck / Institute for Ion Physics and Applied Physics; M. Bunge, Justus Liebig University Giessen / Institute of Applied Microbiology; R. Düring, Justus Liebig University Giessen / depending on nanocatalyst types and concentrations, different dehalogenation products and altered dehalogenation rates were observed. To assess the stability of the nanocatalysts and to estimate their durability under environmental conditions, the approach was expanded to experiments using different nanopalladium fractions, synthesis routes (including laser ablation and microbial “bioPalladium” synthesis), directed catalyst poisoning using reduced sulfur compounds, and oxidative catalyst inactivation. Further tests involving changes of reaction parameters are under way and will demonstrate the high potential of Pd(0) nanocatalysts for clean-up of drinking water, aquifers and specific municipal and industrial effluents, as well as the suitability of PTR-ToF-MS to assess the pertinent transformation and detoxification reactions.

WE183 Triclosan transformation products: Environmental risk evaluation
M.J. Bock, T.R. Barber, P.C. Fuchsman, ENVIRONMENT International Corp.; M. Capdeville, Colgate-Palmolive Company The use of personal care products has led to an increase in the numbers and quantities of contaminating substances. While antimicrobial and cosmetic chemical transformations may occur during wastewater treatment, and the creation of different chemical species during treatment and post discharge can present additional challenges in assessing environmental impacts. Triclosan is a broad-spectrum antibacterial compound that is widely used in a variety of clinical and household applications.

WE182 DEVELOPMENT OF ANALYTICAL METHOD FOR CMIT AND MIT IN WET WIPES
J. Heo, Pusan National University / Civil and Environmental Engineering; U. Kim, Pusan National University / Department of Civil and Environmental Engineering; J. Oh, Pusan National University
In 2012, the humidifier disinfectant had become a serious social problem in Korea due to the 78 deaths including the infants caused by the misuse of humidifier disinfectants. Among the many humidifier disinfectants, the CMIT (chloromethylisothiazolion) and MIT (methylisothiazolione) are the representative chemicals and widely used in products like the liquid type of cleaner, washing materials and shampoo etc. They were known to cause skin irritation and contact allergens (Fewings J. and Menne T. et al., 1999) as well as inhalation toxicity (EPAT738-R-98-012, 1998). Even though their frequent use in living environment, limited studies on the toxic effect on human or the occurrence of these chemicals in environmental were performed. Therefore, in this study we aimed to develop an analytical method for CMIT and MIT in wet wipes which are frequently used for infants, using the Liquid chromatography/Mass spectrometry. In order to set up the efficient pretreatment method, ultrasonic associated extraction (UAE) method and direct injection method were tested using the liquid fraction of commercial wet wipes). For evaluation of extraction efficiency, 1000 ng of CMIT and MIT and internal standard (Atrazin-d5 and OIT-d5) were spiked in wet wipes liquid samples. The extraction efficiency of CMIT and MIT for UAE method was 77–102% and 87–110%, respectively, and the extraction efficiency of CMIT and MIT for direct injection method was 50–170% and 19–50%, respectively. The internal standard average recoveries of direct injection and UAE method were 54% and 72%, respectively. Consequently, UAE method was chosen for wet wipes pretreatment method in this study. For validation of this method, accuracy and precision were obtained from the experiment of seven replicate wet wipe samples spiked 1 ng of CMIT and MIT, and those accuracy (precision) of CMIT and MIT were 98.6 (4.1%) and 118.9 (3.1%), respectively. Method detection limits (MDLs) were quite lower than the previously reported analytical methods (chloromethylisothiazolion) and MIT (methylisothiazolione) are the representative chemicals and widely used in products like the liquid type of cleaner, washing materials and shampoo etc. They were known to cause skin irritation and contact allergens (Fewings J. and Menne T. et al., 1999) as well as inhalation toxicity (EPAT738-R-98-012, 1998). Even though their frequent use in living environment, limited studies on the toxic effect on human or the occurrence of these chemicals in environmental were performed. Therefore, in this study we aimed to develop an analytical method for CMIT and MIT in wet wipes which are frequently used for infants, using the Liquid chromatography/Mass spectrometry. In order to set up the efficient pretreatment method, ultrasonic associated extraction (UAE) method and direct injection method were tested using the liquid fraction of commercial wet wipes). For evaluation of extraction efficiency, 1000 ng of CMIT and MIT and internal standard (Atrazin-d5 and OIT-d5) were spiked in wet wipes liquid samples. The extraction efficiency of CMIT and MIT for UAE method was 77–102% and 87–110%, respectively, and the extraction efficiency of CMIT and MIT for direct injection method was 50–170% and 19–50%, respectively. The internal standard average recoveries of direct injection and UAE method were 54% and 72%, respectively. Consequently, UAE method was chosen for wet wipes pretreatment method in this study. For validation of this method, accuracy and precision were obtained from the experiment of seven replicate wet wipe samples spiked 1 ng of CMIT and MIT, and those accuracy (precision) of CMIT and MIT were 98.6 (4.1%) and 118.9 (3.1%), respectively. Method detection limits (MDLs) were calculated by multiplying three times of standard deviations of seven samples, and the MDLs for CMIT and MIT were 0.013 and 0.011 mg/kg, respectively. Those MDL values were considerably lower than the previous reported analytical methods using LC-UV/DAD (e.g., CMIT; 15 mg/kg, MIT; 5 mg/kg) (KATS, 2012) Keywords; CMIT/MIT, wet wipes, UAE, LC/MS Acknowledgement – This work was funded by a grant from the Korean Ministry of Environment (MOE) through "The Environment Health Action Program (2012001370008)".

WE183 Microbial fingerprinting of an improved strategy to assess chemical persistence at the water-sediment interface
C. Islas, Fraunhofer IME / Environmental and Food Analysis; C. Diaz, Fraunhofer IME; P. Shrestha, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; D. Herrendorf, Fraunhofer IME – Institute for Molecular Biology and Applied Ecology / Ecological chemistry
The degradability of chemicals in different environmental compartments is one of the major determinants of their environmental fate and therefore plays a crucial role in regulatory decision making. In the study of chemicals degradability, simulation tests present several uncertainties with regard to performance, evaluation and data interpretation. Therefore, our work aims at the photodegradation in four simulation tests to see how influences degradation of four well studied substances. Two natural sediments, with different textures, were selected to simulate the biodegradation of different test substances (Aniline, Pyriproxyfen, Voriconazol, Celecoxib). For every sediment/test substance, four tests were conducted in parallel including: a setting according OECD 308, OECD 308 modified (with a thinner, ideally fully aerobic sediment layer), OECD 309 and OECD 309 modified (with higher sediment content). The microbial diversity of the different settings was studied with polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Eubacterial primers were used, in both, qRT-PCR and DGGE (GC clamped primers). Additionally, specific primers

photoionization (APPI) source offers a new step forward the multiclass analysis of PCPs, as was found to be useful to analyse sunscreenss and fragrances from lake sediment samples in a previous study by Chiaia-Hernandez et al. [4]. In the present study we expand the applicability of APPI to analyse a selected group of PCPs in biological samples. Fish samples were extracted by pressured liquid extraction (PLE). A purification step consisting in a solid phase extraction (SPE) was carried out afterwards [5]. The samples were prepared to further analysis with high performance liquid chromatography photoionization-triple quadrupole (HPLC-APPI-Quot-ToF/MS/MS). The method was validated and then its applicability was tested through the analysis of some fish samples. The performance of the method along with the levels of PCPs observed in the collected fish samples will be presented. Acknowledgement - The authors acknowledge the research funding from the National Institute of Agricultural Science and Technology (NIAST) of Korea, the National Research Foundation of Korea (NRF) through the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (20160361930) and the Ministry of Environment (MOE) through "The Environment Health Action Program (2012001370008)".
toxicity. In fact, the triclosan-derived dioxin congeners are rapidly metabolized and do not bioaccumulate in fish, so the potential risk to mammals, birds, and fish is minimal. Thus, although they share the name “dioxin” with certain highly toxic compounds, the dioxin congeners derived from triclosan are not of significant environmental concern.

**WE168 Monitoring of Benzenes, Toluene, Ethylbenzene and Xylene (BTX) Residues in Arable Lands around Oil Reservoir**

B. Park, RDA / Chemical Safety; S. Lim, J. Kim, G. Choi, NAAS RDA

The benzenes, toluene, ethylbenzene and xylene (BTX), which are volatile aromatic hydrocarbons and main constituents of gasoline, are neuro-carcinogenic organisms. The presence of BTX in groundwater is of significance in evaluating the maximum permissible allowable level of BTX in arable soil to 1, 20, 50 and 15 mg kg\(^{-1}\), respectively. To understand an arable soil contamination by BTX, we collected 92 samples from the arable lands around oil reservoir, and analyzed the BTX residue using a GS-MS with head-space sampler. A linear correlation between BTX concentration and peak areas was detected with coefficient correlations in the range of 0.8207-0.9995. The method LOQ of BTX was 0.002, 0.014, 0.084, and 0.038 mg kg\(^{-1}\), respectively. Recoveries of 0.5mg kg\(^{-1}\) BTX were found to be 73.7-96.9%. The precision was reliable since RSD percentage (0.7-7.5%) was below 30, which was the normal percent value. Also, BTX in all samples were detected under the LOQ. These results showed that the investigated arable soils around airport and oil reservoir in Korea were not contaminated by oils.

**WE187 A Design of Experiment Approach for Optimizing LC-HR/MS Data Acquisition and Processing on Environmental Contaminants**

M. Hu, The Helmholtz Centre for Environmental Research - UFZ RWTH Aachen University / EffectDirected Analysis; M. Krauss, W. Brack, T. Schulze, Helmholtz center for environmental research - UFZ / EffectDirected Analysis

PCP liquid chromatography-high resolution mass spectrometry (LC-HRMS) is a technique commonly applied in multi- and non-target screening of environmental contaminants. Evaluation of LC-HRMS screening data typically involves peak picking software. The performance of this kind of software and thus the number of identified peaks may depend very much on parameter settings but also on sample preparation and chemical analysis itself. However, the performance of such software has not been widely assessed so far. Therefore, sample preparation, LC-HRMS analysis and peak picking with MZmine 2 software was rigorously evaluated using water extracts spiked with a known set of environmental contaminants at environmentally relevant concentrations. To this end, a strategy for optimal parameter setting was developed. A Design of Experiment (DoE) approach is proposed: (i) D-optimal design was applied for optimizing parameter setup of sample preparation and LC-HRMS analysis (reverse phase LC on a C18 column, coupled to a LTQ Orbitrap XL instrument); (ii) Central Component Face (CCF) design was used to generate different combinations of parameter settings for MZmine 2. The optimized procedure was applied on water samples from Saale catchment in Germany. The results indicate that data acquisition parameters play key roles for data processing. For example, a reduction of cycling time of Orbitrap MS within a reasonable domain significantly increased the number of peaks that could be deconvoluted by MZmine 2. Since lower cycling time improves peak shape, more peaks fulfill the criteria of deviation parameter in MZmine 2. Furthermore, the optimal setting of the MZmine 2 parameters increased the number of peaks by 20% compared to the worst setting. The DoE approach was found to be very useful for optimizing data evaluation data evaluation processes which involve parameters tuning. We demonstrate that MZmine 2 under optimal parameter settings can significantly enhance the throughput of analysis and detect more than 75% of the peaks that can be manually identified both in spiked and native surface water samples.

**WE188 Combining Equilibrium Sampling with Non-Targeted Analysis of Hydrophobic Complex Mixtures in a Complex Matrix**

**K. Knudsmark Jessing**, University of Copenhagen / Department of Plant and Environmental Sciences; J. H. Christensen, University of Copenhagen; P. Mayer, Technical University of Denmark / Department of Environmental Engineering

Risk assessment of complex mixtures is a serious challenge. It is most often based on one-target chemical analysis and one-compound toxicity tests of priority pollutants. However, this approach is meaningless if key contaminants are not known beforehand or the measured compounds do not exert the observed toxicity. A new approach is to use a prediction model based on known contaminations, yielding total pollutant concentrations that do not reflect the actual contaminant exposure of organisms. In this study we combine two novel strategies to 1) enrich yielding total pollutant concentrations that do not reflect the actual contaminant exposure of organisms. When equilibrium partitioning between sludge and PDMS is reached, hydrophobic compounds in the PDMS are back-extracted. This technique provides some unique features for the subsequent instrumental analysis:

- the compounds are enriched by orders of magnitude in the polymer; typical interferences such as humic acids are excluded, and clean up steps largely omitted.
- In 2) non-targeted fingerprint analysis of the chemical activity in these extracts are then performed using comprehensive multidimensional gas-chromatography quadruple-time-of-flight with electron and negative chemical ionization (GC × GC-QTOF-ESI/NCI), providing high peak capacity and excellent separation. This combination also provides the possibility of identification of compound groups in the sewage sludge. Data processing is performed using peak deconvolution algorithms together with pixel-based analysis. The processed data is analyzed with principal component analysis (PCA) to characterize the chemical composition of the sludge samples and determine the main relative differences in the hydrophobic chemical fingerprints. The results presented are supported by total calibration and provide new insight of chemical activity and composition of complex mixtures in complex matrices – the first step towards better risk assessments.

**WE189 Analytical and statistical approaches to preselect relevant organic compounds in the non-target screening by coupling passive sampling and high resolution mass spectrometry: application to groundwater**

C. SOULIER, Université Bordeaux 1 / Laboratory Division; C. Berho, a. togola, BRGM / Laboratory Division

One of ongoing challenge is to protect and preserve water resources. This involves an increased monitoring and the characterization of micropollutants, emerging trend plots and their toxicities of contaminants for the identification. The samples are mostly released by wastewaters discharge into surface waters and then into other environmental compartment. All these compounds are present in complex mixture at low concentration, implying the need of specific analytical methodology to identify them. The aim is to support public policy development of highlighting and identifying compounds of interest present in groundwater. The main difficulties for the implementation of monitoring are low concentration levels and mix of pollutants from various sources. Therefore there is a strong interest to combine passive sampling and high resolution mass spectrometry (HRMS). On the one hand passive sampling tools allow accumulating compounds during exposure and improving the sensitivity by their integration abilities. The Polar Organic Chemical Integrative Sampler (POCIS) were used to sampling polar and semi polar compounds. On the other hand HRMS allows detecting and identifying organic compounds. The high resolving power, mass accuracy and the sensitive full spectrum acquisition of this technique are the key points for organic compounds screening. Different groundwater impacted by agricultural, urban or rural pressures were investigated and sampled during several months. Grab and passive sampling was employed and analyzed by LC-QTOF. To process data different approaches were investigated. The first approach is based on research from compounds listed on homemade database (around 450 with experimental data on our system as retention time, exact masses for molecular and fragment ions). The non-targeted screening was applied using statistical tools such as principal components analysis (PCA) with direct connections between original chromatograms and ion intensity. Trend plots and their toxicities of contaminants for the identification. This approach allows making comparison of samples and giving multidimensional visualization of chemical patterns as molecular fingerprint and highlighting recurrent or specific peaks of each site. The identification of relevant signal was partially succeeded by using different database such as Norman Mass Bank or Chemspider. The workflow used allows identifying compounds never revealed in these sampling sites.

**WE190 FAST AND COMPREHENSIVE ANALYSIS OF PESTICIDES IN SURFACE WATERS BY UHPLC-HIGHER RESOLUTION & HIGH MASS ACCURACY HYBRID LINEAR ION-TRAP ORBITRAP MASS SPECTROMETRY**

C. Nannoni, V. Boti, C. Tsotsi, University of Ioannina / Department of Chemistry; N. Mantzos, TEI of Epirus / Department of Agricultural Technology; I. Konstantinou, University of Patras / Department of Environmental Natural Resources Management; G. Patakouitos, G. Karras, TEI of Epirus / Department of Agricultural Technology; T. Albani, University of Ioannina / Department of Chemistry

The widespread use of pesticides for agricultural and non-agricultural purposes has resulted in the presence of their residues in various environmental matrices. Pesticide contamination of surface waters has been well-documented worldwide and constitutes a major issue that gives rise to concerns on a local, regional, national and global scale. Pesticides' activity is typical based on their reactions, which are not necessarily specific, yielding total pollution concentrations that do not reflect the actual contaminant exposure of organisms. In this study we combine two novel strategies to 1) enrich hydrophobic compounds so that the obtained extracts reflect the available exposure in the matrix and 2) obtain non-targeted chemical fingerprints of these extracts. Sewage sludge is the studied matrix. In 1) equilibrium sampling is performed with jars coated on the inside with micrometer thin silicone PDMS. This polymer is chosen as it provides enrichment for hydrophobic compounds with biological relevant hydrophobicity. When equilibrium partitioning between sludge and PDMS is reached, hydrophobic compounds in the PDMS are back-extracted. This technique provides some unique features for the subsequent instrumental analysis:

- the compounds are enriched by orders of magnitude in the polymer; typical interferences such as humic acids are excluded, and clean up steps largely omitted.
- In 2) non-targeted fingerprint analysis of the chemical activity in these extracts are then performed using comprehensive multidimensional gas-chromatography quadruple-time-of-flight with electron and negative chemical ionization (GC × GC-QTOF-ESI/NCI), providing high peak capacity and excellent separation. This combination also provides the possibility of identification of compound groups in the sewage sludge. Data processing is performed using peak deconvolution algorithms together with pixel-based analysis. The processed data is analyzed with principal component analysis (PCA) to characterize the chemical composition of the sludge samples and determine the main relative differences in the hydrophobic chemical fingerprints. The results presented are supported by total calibration and provide new insight of chemical activity and composition of complex mixtures in complex matrices – the first step towards better risk assessments.
2000 [3] and has now reached the status of a mainstream mass spectrometry technique. In this study, the analysis of a variety of pesticide residues by means of high mass accuracy hybrid linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap-MS) is investigated. The identification of the positive ions is accomplished with the data from accurate masses of the target ions, based on the full-scan exact mass measurement of [M+H]+ ions, along with retention time data and characteristic on-source fragment ions. The above-mentioned procedure was evaluated in order to be applied for the estimation of the pollutant load and its seasonal distribution in natural waters of the Prefecture of Epirus (Araeots and Louros rivers, Amvrakikos gulf), N.W. Greece. The results obtained confirm that high-resolution mass spectrometry is a helpful and reliable tool for the identification and quantitation of pesticide residues, providing at the same time high accuracy and speed. The method is implemented through the Operational Program “Education and Lifelong Learning” and is co-financed by the European Union (European Social Fund) and Greek national funds.

REFERENCES

WE191
Development of a multiresidue method for the analysis of novel flame retardants in environmental samples using gas chromatography coupled to Time-of-Flight mass spectrometry (GC-ToF)  
M. Lauzent, LPTC-EPOC, University of Bordeaux / UMR EPOC LPTC; P. Labadie, University of Bordeaux / UMR EPOC Equipe LPTC; K. Lemenach, University of Bordeaux / UMR CNRS EPOC LPTC; E. Geneste, Université de Bordeaux / UMR EPOC LPTC; H. Budzinski, University of Bordeaux / UMR EPOC Equipe LPTC.

A review of the phasing out or the voluntary removal of polybrominated diphenyl ether (PBDE) commercial formulations, an increasing number of alternate flame retardants are being used. In the present study, a multiresidue method based on gas chromatography coupled to Time-of-Flight (GC-ToF) mass spectrometry was developed for the determination in environmental samples of 11 alternate brominated flame retardants: bis(2-ethyl-hexyl)teratromethaphthalate (BEHTBP), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (ETBB), tetrabromoethylhexylcyclohexane (aBTECH), octabromotriethylbenzylidane (OBIND), pentabromotoluene (PBT), pentabromobenzene (PBB), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenyl ether (DBDPE), hexabromocyclcopentenyl-dibromobicyclooctane (HCBCDO), hexabromobenzene (HBB), 2,3-dibromobutyl-cyclopentanone (DBPC), and dibromochlorinated diphenyl ethers (DDE, DDT, and DDD). The proposed method allows the analysis of samples with high compound diversity of wastewater, it is essential to take into consideration the complexity of the spectra and the number of elemental compositions detected. The performances of the systems were assessed using both electron ionisation (EI) and electron capture negative chemical ionisation (ECNI). The results show that ionisation method is an influential method and the method was fully validated. We will illustrate the abilities and the performances of the method with an application on the determination of flame retardants in a variety of environmental samples such as fish tissues or sediments collected in French estuaries and riverine ecosystems.

WE192
Tidal Effects on the Occurrence of Pharmaceuticals in the River Thames using Semi-Targeted Liquid Chromatography-High Resolution Mass Spectrometry and in silico Data-Mining Tools  
K. Munro, Analytical and Environmental Science; A. Edge, C.P. Martins, Thermo Fisher Scientific; D.A. Cowan, Kings College London / Drug Control Centre; L. Barron, Kings College London / Analytical and Environmental Science.

The Thames Tideway runs through the heart of London. This section of the River Thames contains a large structural backwater (sewage overflow) CSO vents, which form part of a Victorian sewage system that is struggling to cope with the demands of this modern day city capital. In order to flexibly assess the breadth of pharmaceutical contamination in the Tideway it is necessary to move away from traditional targeted analytical methods. Using high resolution mass spectrometry (HRMS) and in silico data-mining/prediction tools, it is possible to comprehend the overall target (or non-target) approach; i.e. ‘semi-targeted’ analysis. The use of artificial neural networks (ANNs) for the prediction of chromatographic retention time in combination with HRMS scanning software holds potential to simplify the challenging identification process. Presented herein is the application of this approach to assess the effect the river tide had on the occurrence of pharmaceuticals concentrations and occurrence within the Thames Tideway over a 12-hour period, and for the first time, the use of predicted retention times to potentially identify the presence of new contaminants in the River Thames. A number of structurally diverse model species were used to develop a broad targeted quantitative analytical method. The use of HRMS permitted simultaneous target analyte quantification and qualitative semi-targeted analysis of analyte content. An optimised automated data-mining method was used to shortlist potential additional contaminants. An optimised proportion of chromatographic retention times for > 80 compounds spiked into river water were used to train, verify and blind test ANN suitability for retention time prediction in matrix. Results presented show that pharmaceutical concentration fluctuations were compound specific, with some compounds (carbamazepine) showing increasing concentrations in correlation with river tide due to systematic unknown retention constant throughout the sampled time period. The use of in silico tools resulted in several unknown species being shortlisted and identified, with a selection later confirmed with a reference standard. Overall, the potential of semi-targeted screening has been highlighted, allowing simultaneous identification of unknowns while allowing traditional targeted quantification, enhancing the analysis of the aqueous environment.

WE193
Characterization of influent and effluent wastewaters by high-resolution mass spectrometry: Understanding the composition of recalcitrant chemicals  
N.V. Heuett, Florida International University / Department of Chemistry and Biochemistry; P.R. Gardinali, Florida International University / Chemistry Biochemistry and SERC.

In order to fulfill human “needs” in our rapidly changing society, a great variety of products ranging from pharmaceuticals and personal care products (PPCPs), antibiotics, drugs of abuse (illicit and prescription), artificial sweeteners, nanomaterials, disinfection byproducts, sunscreens, pesticides, to name a few; are being manufactured, consumed, used, and disposed of, on a daily basis in households and industrial settings. This massive collection of compounds (in their unchanged form or as transformation products) is not only able to reach aquatic environments, but in some cases persist and potentially inflict detrimental effects upon it. In South Florida, wastewater treatment plants provide typical secondary treatment, followed by disinfection, and release through oceanic outfalls, deep well injection, and/or irrigation. In the South Florida environment, it is essential enough to remove all compounds, turning into a relentless source of contamination. To better understand compound diversity of wastewater, it is essential to take advantage of high-resolution instruments such as the Orbitrap, which provide great sensitivity, optimal resolution, and high accuracy. An initial assessment was performed using a reclaimed water sample (20-liter). Extraction of three-4 liter subsamples using basic liquid-liquid extraction techniques at three different pH’s (3.5, 7.0, and 9.6) was carried out; and extracts were then fortified with a set of 24 isotopically label standards for quantification purposes. A combination of target and non-target screening was performed on the extracts using LC-ESI-HRMS in both positive and negative mode. A QExactive Hybrid Quadrupole-Orbitrap HRMS was used in full scan mode at 140,000 RP for molecular characterization. Preliminary results provide a clear indication that a simple extraction process is capable of generating molecular information of complex mixtures like treated wastewater. The complexity of the spectra and the number of elemental compositions detected are related to both the extraction pH and the ionization technique. Kendrick, DBE vs. carbon number, and van Krevelen diagrams were used to identify compound families with elemental compositions containing heteroatoms like sulfur, oxygen, nitrogen, chlorine, and fluorine.

WE194
Detection of Designer Drugs and Relevant Metabolites in Wastewater Samples Using High Resolution Mass Spectrometry: a connection between target, semi-target and unknown-unknown analyses  
M. Pruyn, Florida International University FIU / Department of Chemistry and Biochemistry; P.R. Gardinali, Florida International University / Chemistry Biochemistry and SERC.

New designer drugs are emerging in the illicit drug market faster than they could be detected or managed. Due to minor modifications made to the parent compound and the multiple options when transforming a common chemical backbone large number of novel compounds are introduced to the consumers regularly mainly driven by public perception and underground marketing. Known designer drugs can be detected as target compounds once enough evidence on their use is gathered. Novel homologues may also be detected using known fragmentation patterns of the target compound. A multitude of unknown-unknown workflows relying on high resolution mass spectrometry coupled to in-silico predictions and statistical interpretations. The aim of this study was to detect all known designer drugs, their potential metabolites and transformation products and potentially related structures in wastewater streams from influents to effluents. Wastewater samples were collected and stored in the dark at -20 °C to prevent degradation. Sample treatment considered a modification of standards and pre-concentration using offline or online SPE. High resolution mass spectrometry was conducted using the QExactive Orbitrap at resolutions above 70,000 with a HESI II ionization source operated in both positive and negative mode. The raw data was analyzed using a basic screening method in TraceFinder EFS using an in-house database of designer drugs previously described in the available literature. The vast majority of these compounds have never been reported in environmental samples. Chromatographic peak shapes, isotope ratios, S/N limits and retention times were used to conduct the initial screening. Preliminary assessment of archived samples yielded multiple designer drugs candidates, such as 4'-methyl-o-pyrrolidinohexanophene (MHP).
Do glucuronidated pharmaceuticals contribute to transformation products in the aquatic system?" B. Zona, IDAEA-CSIC / Environmental Chemistry; A. Delgado, IQAC-CSIC / RUBAM; S. Perez, IDAEA-CSIC / Environmental Chemistry; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research

The present work gives insight into the behaviour of pharmaceutical glucuronides which are excreted from the human body and enter into the aquatic environment via WWTP. Initially, a retrospective suspect-screening method for the determination of the transformation products of the potent anti-epileptic lamotrigine and its human metabolite, the lamotrigine glucuronide, was suggested that lamotrigine undergoes unusual transformations. This was discovered by application of a generic solid-phase extraction (SPE) method to a wastewater and surface samples and their subsequent analysis with high resolution mass spectrometry (HRMS). Biodegradation reactors were amended with mixed liquor and inoculanted with lamotrigine at 50 ng/mL in order to identify the transformation products of lamotrigine and further compare with the transformation products already detected in the analysed real WWTP samples. Experimental lab-scale bioreactors at neutral pH and lamotrigine at environmentally relevant concentrations proved that lamotrigine was resistant to biodegradation with less than 20% elimination after one week. Discarding the possible TP formation from lamotrigine, its human metabolite, the lamotrigine glucuronide, was also degraded in the batch reactors. Since lamotrigine is extensively metabolized in liver, its glucuronide could be an important contributor to lamotrigine derived compounds in the environment. In these batch reactors, it was found that the glucuronide formed two transformation products which were previously detected in the real environmental water samples. The main TP was a direct biodegradation product (the reaction was an oxidation of the glucuronide conjugate). The other TP was formed from deconjugation of the hydrolysed glucuronide. The reaction preceding the deconjugation step is hydrolysis of the original glucuronide, which is exclusively governed by the abiotic pH change. Finally, in order to understand better the reactions glucuronides undergo, a selected number of various glucuronides were biodegraded as well. They are classified in three groups. The first group contains N-C glucuronides which have no known glucuronidation patterns. The second C-C glucuronides and the third class are the C-acyl glucuronides (connected to an acid). In total 11 additional glucuronides were degraded (5 N-C, 3 C-C and 3 C-acyl). Reactions of oxidation, migration and deconjugation were observed as well.

**WE198** Method development for non-target screening of sewage sludge using gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS)

C. Veenas, Umea University / Chemistry; P. Haglund, Department of Chemistry

**Keywords:** Non-target screening, sewage sludge, method development, GC-HRMS in strongAbstract

More than 100,000 chemicals are present in the technosphere and 30,000 are selected as hazardous by the chemicals”. Sewage treatment plants (STPs) are used to remove nutrients, but also some metals and organic chemicals, from urban waters and create a less contaminated effluent. Consequently, STPs form a link between the technosphere and the environment. A by-product of the sewage treatment process is sewage sludge—a solid product that contains nutrients as well as pollutants. These nutrients make sewage sludge attractive for applications as fertilizer on agricultural fields, provided that the contaminant levels are not too high. In order to be able to investigate pollutants occurring in sewage sludge an analytical method for comprehensive non-target screening is needed. Up till now there is no method existing that allows a full screening of sewage sludge for a wide variety of compounds with different properties, which is the ultimate goal of this project. The current study aims to develop a non-discriminating sample preparation method for gas chromatography - mass spectrometry (GC-MS) analysis. Pressurized liquid extraction (PLE) was used for extraction, with in-line and/or off-line clean-up. In the in-line approach silica gel was added to the PLE cell for simultaneous extraction and clean-up, whilst the off-line approach involved clean-up by gel permeation chromatography. Analysis was performed by GC high-resolution MS in both cases. Preliminary results indicate that the in-line clean-up can be used for analysis of established contaminants as well as new emerging contaminants.

**WE199** Determination of priority and emerging target and non-target contaminants in aqueous samples by atmospheric pressure gas chromatography-time-of-flight-mass spectrometry (APGC-ToF-MS)

M. Piñatdo-Herrera, Universidad de Cádiz (Spain) / Department of PhysicalChemistry; E. Gonzalez-Mazo, University of Cadiz / Physical Chemistry; P. Lara-Martin, University of Cadiz (Spain)

**Keywords:** GC-APGC-ToF-MS, target screening, sewage sludge, method development, determination, and validation of a multi-residue method for the simultaneous determination of 102 contaminants, including fragrances, UV filters, repellents, endocrine disruptors, biocides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and several types of pesticides in aqueous matrices. We have focused our attention on the possibilities offered by APGC-ToF-MS for the identification and quantification of target and non-target micropollutants in water samples after stir bar sorptive extraction (SBSE). APGC ionization occurs by charge transfer (M+) or protonation (M+H+), using a reagent gas (nitrogen) and lower energy than the traditional electronic impact (EI) so fragmentation of the molecular ion is lower. When compared to EI, we could observe that the latter is often a better option when...
working with very stable compounds such as PAHs, which require higher ionization energies for ionization and fragmentation. On the other hand, better results were obtained using APGC for more labile compounds (i.e. fragrances). Moreover, APGC allows for a progressive fragmentation by applying different cone voltages and can be a very powerful tool to predict and confirm fragmentation patterns (especially if specific software such as Mass Fragment is available), therefore enabling the correct identification of some target analytes (i.e. macroyclic fragrances) which were not properly determined by EI. Additionally, coupling APGC to ToF mass analyzers may improve not only the sensitivity for some compounds but also the mass resolution achieved in full scan mode by more conventional single quadrupole mass analyzers. Thus, the combination of accurate mass measurement, retention time and isotope models (comparison between original acquired mass spectrum and the theoretical isotope model) can be used as additional confirmation criteria during analysis of target (and non-target) compounds. We applied this technique to several environmental aquatic matrices (seawater, sewage effluent, river water and groundwater), determining the concentrations of 21 out of 102 analytes. OTNE, galaxolide, tonalide, benzophenone-3, triclosan, and homosalate were detected in all samples (up to 18.2 μg L⁻¹). Occurrence of several non-target compounds such as dibenzyl ketone (fragrance), dibutyl phthalate (plasticizer) or tributyl phosphate (flame retardant) was also confirmed.

**WE200** Combining chemical and biological screening: a tool for identifying novel priority pollutants in complex environmental mixtures

M. Larsson, Orebro University / MTM research center Department of Science and Technology; O. Westman, Akademin för naturvetenskap och teknik; J. Hagberg, MTM Research Centre, Orebro University / Dept. of Science and Technology; M.M. Lam, RWTH University Aachen / mtm Research center Department of Science and Technology; B. van Bavel, MTM Orebro University; M. Engwall, Orebro University / MTM research center Department of Science and Technology; M. Tarr, HUPLC–ESI-Q-Orbitrap–HRMS laboratories.

Studies have shown that traditional monitoring methodology of using chemical analysis of a small number of pollutants to determine the degree of contamination in environment matrices, like soil, dust and sediment, usually overlooks potential contaminants present in the samples. The objective of the present study is to establish an analytical method to screen for organic pollutants in environmental matrices with the goal to identify (emerging) pollutants posing a hazard to ecosystems and human health. A combining of chemical and biological screening is used to evaluate the occurrence and aryl hydrocarbon receptor (AhR)-mediated toxic potential (cocktail effect) of PAHs and related compounds in complex environmental matrices. So far, two different matrices have been tested; samples from an unspecified lake and soil sediment. The AhR-mediated potency of these compounds can then be tested individually in the bioassay. Risk assessment of contaminated areas would be easier and safer if adequate marker compounds were available. Analysis of these markers along with the monitoring pollutants may provide a broader picture of the contamination.

**WE201** Non-target Time Trend Screening (NTTS) for Identification of Novel Xenobiotics in Human Blood

M. Plassmann, University of applied environmental science; J.P. Benskin, Stockholm University / Environmental Science and Analytical Chemistry

Most methods for screening environmental contaminants target individual chemicals or chemical classes using highly specific analytical approaches. Despite their utility for low-level detection and quantification, these methods often overlook novel contaminants or transformation products which may pose a risk to humans and wildlife. Recent advances in mass spectrometry and chemometrics have led to development of ‘non-targeted’ screening approaches, in which samples are analysed without a priori knowledge of the contaminants of interest. These methods are now validated against samples with known xenobiotics and have been analysed by both GC-MS (SIM and full scan) and H4IEE-luc bioassay analysis. Statistical multivariate analysis will be used to characterise the samples and link GC-MS full scan spectra (>1400 markers) and H4IEE-luc bioassay results, that is, look for relationships between compound composition and AhR-mediated activity i.e., potential chemical markers for toxicity. The AhR-mediated potency of these compounds can then be tested individually in the bioassay. Risk assessment of contaminated areas would be easier and safer if adequate marker compounds were available. Analysis of these markers along with the monitoring pollutants may provide a broader picture of the contamination.

**WE202** Novel one-step centrifugal sensor system for the detection of cyanobacterial toxin Microcystin-LR

J. Fitzgerald, Dublin City University / School of Chemical Sciences; I. Maguire, B. Heery, C. Swankire, C. Murphy, J. Ducrée, R. O’Kennedy, Dublin City University; f. Regan, Dublin City University / Chemical Sciences

Globally, the most prevalent toxic algae is, in blooms from fresh and brackish waters, are the cyclic peptide toxins of the microcystin family. The need for on-site algal-toxin monitoring has become increasingly urgent due to the amplified demand for fresh-water and for ‘toxin-free’ shellfish and fish stocks. Along with routine testing of shellfish stocks for biotoxins including microcystin, the EU also require routine monitoring for the presence of the causative algal species. Herein, we describe a novel, Lab-On-A-Disc (LOAD) platform which has been developed to assess microcystin toxin levels in-situ. Using recombinant antibody technology, the LOAD platform combines immunofluorescence with centrifugally driven microfluidic liquid handling to achieve a next-generation disposable device for high throughput sampling. A low-complexity, ‘LED-Photodiode’ based optical sensing system was tailor-made for the platform, enabling the fast and accurate quantification of microcystin in less than 15 minutes with minimum user interaction and maximum reproducibility. This method provides a low cost diagnostic alternative to the current laborious and costly methods used for toxin monitoring.

**WE203** Braaavo: biosensors for real time monitoring of marine contaminants

J. Sánchez, IDAEA-CSIC / Water and Soil Quality Research Group; J. van der Meer, UNIL; M. Farre, IDAEA-CSIC; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research;

BRAAVO is a FP7-Ocean project which aims to develop innovative solutions for real-time in-situ measurement of high impact and difficult to measure marine contaminants. The BRAAVOO concept is based on unique integration of three types of sensing approaches: nano-immuno, bacterial, and algal biosensors. This robust multi-usage approach ensures the detection of specific priority pollutants (including BTEX, a wide range of alkanes, several polycyclic hydrocarbons, PAHs, and several pharmaceuticals) at environmentally relevant concentrations, and general toxic effects in what will be an effective marine early warning platform. The three biosensors will be miniaturized and integrated into novel chip-based modules which will be located on board of a data buoy and an unmanned surface vessel. Both systems will be energetically autonomous for a period of several weeks while maintaining living cells, ensuring the regeneration of the fluid and collection sample. Real-time and in-situ continously monitored and sent to a permanent base where it can be processed. The system will also allow the collection and storage of large volume samples for re-analysis and confirmation. Several SPME-GC-MS/MS SPE-UHPLC-ESI-Q-Orbitrap-HRMS methodologies have been developed for this purpose. The novel analytical methodologies and examples of their applications will be presented.

**WE204** Photonic nanobiosensors for real time monitoring of chemical contaminants in marine environment

S. Herranz, Nanobiosensors and Bioanalytical Applications Group; J. Sanchez, IDAEA-CSIC / Water and Soil Quality Research Group; M. Farre, IDAEA-CSIC; M.P. Marco, IQAC-CSIC CIBER de Bioingeniería Biomateriales y Nanomedicina CIBER-BBN / Nanobiotechnology for Diagnostics Nbd group; L.M. Lechuga, Institut Català de Nanociència i Nanotecnologia ICN2 CSIC & CIBER-BBN / Nanbiosensors Bioanalytical Applications Group

A technological environment supports a large number of different species of organisms and plays a key role in climate and weather patterns. Moreover, the ocean is one of Earth’s most valuable natural resources, it is used for transportation (both travel and shipping) and it is a major location for leisure tourism. As a result of intensive exploitation there is a slow but steady degradation of the marine water quality. Therefore, actions against ocean pollution must be taken, and numerous monitoring programmes have been established in place by individual countries and international organisations. Therefore, there is a growing need of sensitive field analysis tools which allow to perform real-time on-site sample analysis. In this context, we are developing integrated photonic nano-immunosensors based on a silicon technology with a view to implement a complete lab-on-a-chip platform. In particular, the bimodal waveguide interferometer (BIMW) [1]. In the BIMW configuration, two guided modes of the same polarization interfere in a single waveguide, without the need of a reference arm: light from a coherent source is coupled into a single mode rib waveguide which is transformed into a thicker waveguide supporting two guided modes (fundamental and first order modes), that propagate until the output of the waveguide with different intensity distribution at
the core-cladding interface. As the fundamental mode is more confined than the first order mode, it is less sensitive to the upper cladding parameters and acts as an internal reference [2]. As target, the antifouling paint booster biocide Irgarol 1051 has been selected. The assay is based on a competitive inhibition format in which an irgarol-protein conjugate is covalently immobilized on the sensor surface. We have investigated several factors affecting sensor performance: conjugate nature and sensor immobilization protocol, target concentration, measurement conditions, etc. Preliminary results show limits of detection at the nM level. Further work is being carried out to fully characterize the analytical behaviour of the sensor and apply it to seawater samples analysis. [1] Zinoviev et al., 2011. J. Lightwave Technol. 29:1926-1930. [2] Duval et al., 2012. Lab Chip 12:1987-1994. Acknowledgement - This work has been funded by the TFP (EU, BRAAVO Grant Agreement No 614610). ICN2 acknowledges support from the Severo Ochoa Program (MINECO, Grant SEV-2013-0295).

WE207 Assessment of PLE and QuEChERS methods to determine pesticides in soils, sediments and sludges from Turia River Basin M. Reyes, K. Vasquez, J. Campo, University of Valencia; Y. Pico, University of Valencia / Medicine Preventive

Pesticides have played an important role in the development of the agriculture but most of them can reach the environment via run off becoming a real environmental pollution and human health risk. In this sense, contamination of sediments with agricultural pesticides is still a problem of primary concern, because they are easily accumulable in these matrices. Consequently, there is increasing need for the development of well validated, accurate, time saving, low cost, modern, multicomponent methods. The purpose of this study is to compare two extraction techniques for 50 pesticides in sediments in order to identify a suitable extraction method for the multiresidue analysis of sediments, soils and sludges. The performance of two enhanced extraction techniques, such as pressurized liquid extraction (PLE) and QuEChERS extraction for the determination of pesticides in dry sediments was investigated using liquid chromatography tandem mass spectrometry (LC-MS/MS) in positive ionization mode with an electrospray ionization source (ESI). Separation was carried out on a Luna C18 column (150 x 2.0 mm, 3 μm) using a gradient elution profile with mobile phase consisting of water-methanol, both 10 mM ammonium formate. Both extraction techniques produced acceptable recoveries for the pesticides under study. Mean recoveries for PLE were 68, 72 and 71 % and for QuEChERS were 76, 73 and 82 % for soils, sediments and sludges respectively, and RSD were lower than 20%. QuEChERS procedure was selected because it was advantageous in terms of recoveries and additionally, it was low time consuming, easier and cheaper than PLE. Finally, it was validated, providing LOQs between 1 and 10 ng g⁻¹ dw with the exception of alachlor and metolachlor (25 ng g⁻¹ dw) for all matrices, and applied to determine pesticides in samples taken from Turia River Basin. Acknowledgement: This work has been carried out with the support of the Spanish Ministry of Economy and Competitiveness through the projects Consolider-Ingenio 2010 CSD2009 and CGL2011-29703-C02-02. Keywords: pesticides, pressurized liquid extraction, QuEChERS, monitoring

WE208 Exploring four extraction procedures for the determination of perfluoroalkyl substances in soil, sediments and sludges from Turia River Basin. M. Lorenzo, J. Campo, University of Valencia; C. M. A. Reyes, P. Y. Pico, University of Valencia / Medicine Preventive

The analysis of perfluoroalkyl substances (PFASs) in soils, sediments and sludges is important for understanding the transport, accumulation and fate of these pollutants in the environment. A comparative study of four extraction methods for the determination of PFASs in soils and sediments was performed in order to select the one that provides better recoveries and higher sensitivity. The determination of PFASs (C4–C14, C16, C18 carboxylates, C4, C6–C8 and C10 sulfonates, two C8 sulfonamides and C10 native unsaturated telomer acid) was made by liquid chromatography-tandem mass spectrometry (LC-QqQ-MS/MS). The compared methods were based on extraction with (i) aqueous solution of acetic acid and methanol, (ii) methanol, (iii) digestion with sodium hydroxide (NaOH) in methanol and (iv) ion-pair extraction. The best results were obtained with methanol extraction which recovered a greater number of PFASs and provided recoveries between 69-90% in sediment and 86-93% in soil with relative standard deviations (RSDs) < 31%, and limits of quantification (LOQ) between 0.02-0.45 ng g⁻¹ dw for sediment and 0.01-0.59 ng g⁻¹ soil. The selected method was successfully applied to Segura River sediments and Turia River soil samples. Likewise, the two methods with better results (methanol and aqueous solution of acetic acid and methanol) were also applied to other matrices not proved in this study, such as sewage sludge, to verify their effectiveness. This study demonstrates the presence of PFASs in the Valencian Community Rivers studied, and the problematic of sewage treatment plants where these compounds are not sufficiently eliminated. Acknowledgement: The authors thank the Spanish Ministry of Economy and Competitiveness for the financial support through the projects Consolider-Ingenio 2010 CSD2009, and CGL2011-29703-C02-02, Julian Campo also acknowledges the VAL+Id postdoctoral contract (APOSTD/2014/010) of the Generalitat Valenciana for funding. Keywords: soil, sediment, sludge, perfluoroalkyl substances

WE209 Use of GC/MS Triple Quadrupole instrumentation for Nitrosamine analysis D. Caruana, D. Steninger, H. Huebschmann, Thermo Fisher Scientific
“Classical” nitrosamine analysis was performed for many years by gas chromatography using a thermal energy analyzer (TEA) as detector. Today mass spectrometric methods have increasingly replaced the TEA. EPA method 521 by Munch and Bassett from 2004 provided a suitable GC-MS method based on chemical ionization (CI) using an ion trap mass spectrometer with internal ionization, in contrast to standard quadrupole or ion trap mass spectrometers using a deuterium (external) ion source design. At present current developments in GC-MS triple quadrupole technology deliver enhanced sensitivity and selectivity for small molecule mass range compounds and allow the detection of nitrosamines at very low concentration levels even in complex matrix samples. This is made possible by using a much simpler and standard approach with the regular electron impact ionization (EI) for a very straightforward method for low level nitrosamine analysis. The work documented completes a GC-MS/MS method for routine detection and quantitation nitrosamine compounds. Special focus in the method development process was given to provide a fast, easy to implement routine method with the required sensitivity for monitoring nitrosamine compounds. The final method was evaluated for validation criteria including linearity, accuracy and precision. Method detection limits were determined in the 1 ppb range. Spiked and non-spiked water samples analyzed for low concentrations of target nitrosamines.

**WE210**

**Exploration of suitable alternatives for GC/MS He carrier gas conservation**

D. Cardona, T. Jeffers, T. Albertini, Thermo Fisher Scientific

The impact of increased price and decreased availability of helium continues to drive the need for carrier gas conservation in GC/MS. During sample introduction for volatile organic compound analysis via purge and trap apparatus large amounts of helium are consumed. Various solutions have been attempted to ease the burden of helium shortage including the use of alternate carrier gasses. In our experiments use of an alternate Hydrogen carrier gas was explored as well as use of an innovative He conservation device that limits the flow of helium during sample vaporization in the inlet. The two system configurations were evaluated. The results obtained from the Helium conservation device were compared to results obtained from a system utilizing constant helium flow. Use of hydrogen carrier gas for semi-volatile analysis via $8260$ yielded negative long term system effects and unforeseen reactions in the purge and trap apparatus. The helium conservation device produced equivalent results compared to a system utilizing constant helium flow while maintaining original method settings indicating a suitable solution for helium conservation. Comparative volumes of helium consumed during analysis were also evaluated.

**Recent scientific developments in bioaccumulation research and assessment (P)**

**WE211**

**Regulatory implications of advances in bioaccumulation testing and assessment**


The bioconcentration factor (BCF) is a well-established metric in bioaccumulation assessment. It reflects the uptake of a chemical in an aquatic organism, commonly fish, from water. In most regulatory systems, a very high BCF is considered an indication of an unacceptable risk. However, in the past years, the assessment of bioaccumulation has experienced some developments that are not yet reflected in the regulatory regimes. For instance, the OECD test guideline for bioaccumulation in fish has been revised in 2012 to incorporate also a second method measuring a biomagnification factor (BMF) following dietary uptake. A definitive criterion has not yet been decided on. BCF and BMF are not suitable for moderately and highly hydrophobic compounds, respectively. A suitable screening criterion for hydrophobic compounds is the log $K_{ow}$. However, for other substance groups, such as ionising or amphiphilic substances, specific screening criteria are needed. Although it is the most common metric in bioaccumulation assessment, the BCF in fish may not reflect the whole risk. A number of substances have been found that accumulate highly in terrestrial organisms, with only a moderate bioconcentration in fish, e.g. PFOS. A log $K_{ow}$ between 2 and 5, in combination with a log $K_{ow}$ > 6 has been suggested as screening criteria. Is this a sensible combination of criteria and how should this be reflected in an assessment scheme? Currently, substances with a high potential for accumulation in air-breathing organisms are usually only identified after entering into the environment. Alternative approaches to replace, reduce or replace animal testing in bioaccumulation assessment have been developed. Although it may seem premature to integrate new test methods such as *in vitro* testing into an assessment scheme, this should be considered as early as possible, in parallel to establishing a test guideline, to ensure that the new methods can be used efficiently once methods have matured. This poster aims at showing recent developments in bioaccumulation assessment with regard to their stage of development, regulatory implications and open questions which need to be solved prior to their integration into substance regulations and assessment schemes to improve the quality of bioaccumulation assessment.

**WE212**

**Is bioaccumulation in zebrafish embryos purely log $K_{ow}$ regulated?**

D. Kurth, Helmholtz centre for environmental research - UFZ / Department of EffectDirected Analysis; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis

With the aim of reducing animal testing, many *in silico* approaches have been developed to model bioaccumulation. The development of new methods are supposed to reduce animal testing in bioaccumulation assessment with regard to their stage of development, regulatory implications and open questions which need to be solved prior to their integration into substance regulations and assessment schemes to improve the quality of bioaccumulation assessment.

**WE213**

**A hydrophobicity parameter for surfactants: An estimation of log $K_{ow}$ values for surfactants using RP-HPLC**

J. Hammer, University of Utrecht / IRAS; J. Haftka, University of Utrecht / Institute for Risk Assessment Sciences; J.L. Hermens, Utrecht University / Institute for Risk Assessment Sciences; P. de Vogt, University of Amsterdam / IBED

Anionic and nonionic surfactants are large-scale produced chemicals which can be found in many consumer products and consequently also in the environment as organic contaminants. Therefore, it is important to understand the fate and pathways of these compounds. Sorption to soil or bioaccumulation into organisms of these surfactants is mostly driven by the hydrophobicity of the individual compounds. The octanol-water partition coefficient ($\log K_{ow}$) is a physicochemical parameter used to describe the hydrophobicity of a substance. However, the conventional methods for determining log $K_{ow}$ data with the shake-flask or the slow-stirring method (OECD guidelines 107 and 123) are not applicable to surfactants because of their surface-active properties. Instead, OECD guideline 117 allows for the octanol-water partition coefficient to be estimated by using experimentally determined capacity factors for surfactants and reference substances obtained from a reverse phase HPLC method. In this study, log $K_{ow}$ values for surfactants were estimated by experimentally determining their capacity factors and compare these with capacity factors of reference substances with known log $K_{ow}$ values. Log $K_{ow}$ values were estimated for different nonionic and anionic surfactant groups, including alcohol ethoxylates, alkybenzylalkylaromatics, alkylalkylamines, alkyalkylamides, alcohols and hydrophobicity and toxicity of nonionic and anionic surfactants. **Keywords:** Anionic Surfactants; Capacity Factors; Hydrophobicity; HPLC

**WE214**

**Protein-water partition coefficients of ionic organic chemicals: Serum albumin and muscle protein as model proteins**

L. Oemisch, Analytical Environmental Chemistry; K. Goss, Helmholtz centre for environmental research - UFZ / Department of Analytical Environmental Chemistry; S. Endo, Osaka City University / Urban Research Plaza Graduate School of Engineering

Assessment of the bioaccumulation potential of organic chemicals is required in various regulatory procedures. Currently, the octanol-water partition coefficient is used as screening criteria in most cases. This idea is chiefly based on the assumptions that lipids are the main compartment that accumulates the organic chemicals within the organism and that lipids can act as a reservoir for lipids. These assumptions seem to be acceptable for screening of neutral chemicals. However, for ionic chemicals, neither of the assumptions seem to be valid. An issue is that the protein fraction has been said to have substantial contribution to the accumulation capacity of organisms. Nevertheless, a systematic evaluation has not be conducted for protein-water partition coefficients of diverse organic ions. Moreover, common used property estimation tools are basically for neutral compounds and not transferable to ionic species. In this study, we measured protein-water partition coefficients of series of anionic and cationic organic compounds using an equilibrium dialysis method. Bovine serum albumin and chicken muscle protein were used as model proteins. Our first results for selected
We215 Optimizing Immobilized Artificial Membrane Chromatography for Organic Cations
s.T. droge, Utrecht University / IRAS; S. Mahawat Khan, J.L. Hermens, Utrecht University / Institute for Risk Assessment Sciences

The lipid-water partition coefficient and biotransformation rate constant are for many compounds the main chemical parameters that determine a bioconcentration factor. Immobilized Artificial Membrane (IAM) chromatography columns allow for high-throughput and consistent determination of phospholipid affinities, with medium composition and other system parameters easily controlled. The retention capacity factors (kIAM) for most neutral compounds strongly correlate with liposome partition coefficients. However, the ability of the IAM “Drug-Discovery” column to adequately deal with ionized compounds has been questioned. The few available liposome sorption studies on ionizable compounds have shown that the membrane affinity of the ionic species of a base is often less than a factor of 10 lower than the neutral base species, indicating that “ionic species should not be neglected in predictions of the bioconcentration potential. A substantial fraction of relevant organic compounds are largely ionic in typical environmental conditions as well as physiological conditions, so this strongly hampers the use of IAM as a standard tool to aid environmental risk assessment and pharmaceutical research. The major drawback of IAM is that compounds may undeniably interact with the IAM column material electrostatically due to charged surface moieties that are not related to the phospholipid coating. These moieties comprise weakly acidic free silanol groups and underivatised free propylamine groups. In this study, we explored which medium conditions may be suitable to obtain adequate kIAM values for 6 fully ionized bases, and why. Our findings show that both salinity and pH of the test medium are key parameters that control the additional Coulombic attraction/repulsion of ions to the IAM column material (IAM,PC.DD2). Using common physiological medium results in an overestimation of membrane affinities of about an order of magnitude for fully charged bases, whereas eluent containing pH 7.4 buffers alone (e.g. 10 mM) would result in even higher overestimation. Under optimal conditions, however, IAM-based membrane affinities for fully ionized bases correspond to liposome partitioning data of the ionized species.

We216 Measurements of phospholipid membrane affinity and biotransformation rate constant of ionogenic organic chemicals: Support as refinement for fish bioaccumulation models
Y. Chen, University of Utrecht / Institute for Risk Assessment Sciences; M.T. Jonker, Utrecht University; J.L. Hermens, Utrecht University / Institute for Risk Assessment Sciences; s.T. droge, Utrecht University / IRAS

Bioaccumulation is one of the key factors in terms of evaluating the fate and risk of chemicals in the environment. Under the chemical legislation REACH, adequate estimates of the bioaccumulation potential of thousands of chemicals, of which 50 % are ionic, are required in the recent registration process. This is particularly for ionogenic organic chemicals (IOCs) in fish has been developed (Armitage et al. 2013) and evaluated against empirical bioconcentration factors (BCF). While bioaccumulation data on IOCs are scarce, outliers in BCF-IOCs model predictions are related to the uncertainty in and lack of empirical data for phospholipid membrane affinities and biotransformation rate constants. Charged IOCs behave differently than their neutral form and tend to interact with phospholipids through more complex interactions. Additionally, the phospholipid-water partition coefficient is also not sufficient to predict bioaccumulation potential when substantial biotransformation occurs. This study aims to improve the measurement of both parameters for a series of charged organic acids and bases. Solid-supported liposome membranes (TRANSL) were applied to determine partition coefficients of these selected IOCs at a fixed pH of 7.4. An in vitro method using fish liver S9 fraction was used to measure the decrease of the concentration of the parent compound over time. Biotransformation rate constants were then calculated according to the protein concentration and incubation time. The empirical data can be used to improve estimates of the bioaccumulation potential of the tested IOCs and allow for updates of IOC bioaccumulation model parameters.

We217 Expanding the applicability of a mechanistic mass balance model for estimating the bioaccumulation potential of ionizable organic chemicals in fish
J.M. Armitage, University of Toronto - Scarborough / Department of Physical and Environmental Sciences; T.N. Brown, Dalhousie University / Department of Chemistry; F. Wania, University of Toronto at Scarborough / Department of Physical and Environmental Sciences; D. Mackay, Trent University; J.A. Arnot, Canadian Research & Consulting / Department of Physical Environmental Science

Tools to model the bioaccumulation of neutral organic chemicals (NOCs) in organisms and food webs are well-established in the scientific literature and regulatory practice. More recently, the need to assess the bioaccumulation potential of ionizable organic chemicals (IOCs) has been recognized for ‘B’ categorization, exposure and risk assessment, leading to questions regarding the reliability of available tools for such chemicals. While both regression-based and mechanistic bioaccumulation modeling approaches have been proposed, further development and application are required to build confidence in these tools, particularly for IOCs which are predominantly charged at environmentally-relevant pH (e.g., perfluoroalkylated acids and other anionic surfactants, pharmaceuticals and certain pesticides). The purpose of this study is to present an updated version of a mechanistic mass balance modeling approach for predicting whole body bioconcentration factors (BCF) for many simple and complex IOCs. The revisions include new treatments of mass transfer at the gill surface and new tools for model parameterization. Results include BCF predictions as a function of external water pH for organic acids and bases, comparisons of modeled and measured chemical uptake rate constants across the gill (kG) and illustrative examples demonstrating the importance of explicitly including biotransformation rate constants (kB) in BCF calculations. The performance of newly developed Quantitative Structure Property Relationships (QSPRs) for estimating the sorption capacity of the organism (e.g. membrane-water partition coefficients and distribution ratios) is also assessed. Key data gaps and research priorities for improving the reliability of this modeling tool are also highlighted.

We218 Predicting biotransformation and bioconcentration of organic chemicals in fish
J. Stadnicka-Michalak, EPFL - Swiss Federal Institute of Technology; F. Weiss, Eawag - Swiss Federal Institute of Aquatic Science and Technology / Department of Environmental Chemistry; M. Knobel, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; K. Schirmer, Eawag / Environmental Toxicology

The potential of a chemical to accumulate in organisms is an important criterion in environmental risk assessment. The most commonly applied method to derive bioconcentration factors (BCF) is the Fish Bioconcentration Test, which is technically challenging and resource intensive. Another approach is to predict biotransformation by means of toxicokinetic models. Here, one-compartment models assume that an organism comprises one tank which contains a well-stirred fluid while multi-compartment models, like the PBTK model, take also tissue distribution of a chemical into account. In their simplest design, both these approaches assume partitioning of chemicals by passive diffusion without taking biotransformation into account. A more realistic way, which includes the in vitro biotransformation rate in freshly isolated hepatocytes, has already been introduced. Thus, in our study, we included biotransformation rates in freshly isolated hepatocytes (taken from literature) in both PBTK and one-compartment models to calculate BCFs and compare them to in vitro measured BCFs. In addition, as we hypothesized that chemical biotransformation does not take place only in the liver and moreover can be measured also in cultured fish cells, we measured biotransformation rates in three (liver, gut and gill) rainbow trout cell lines. When both (PBTK and one-compartment) modelling approaches accounted for chemical biotransformation in the liver based on biotransformation rates from freshly isolated hepatocytes, the PBTK model outperformed the one-compartment model for predicting BCFs of all chemicals found in the literature. In many cases, these predictions still overestimated the BCFs, therefore including biotransformation into account is far more realistic. A new approach is to consider the in vitro biotransformation rate in freshly isolated hepatocytes, reduced the BCF from 21000 to 5000 for the one-compartment model and from 9500 to 1700 for the PBTK model, compared to 920 reported for rainbow trout. However, when we applied biotransformation rates from all three cell lines, distinguishing the intestine as important environment-organism barrier, and taking the gill as a representative of other highly perfused tissues in addition to liver and intestine, our PBTK model predicted a BCF of 1060. Thus, our study shows that building a virtual fish from different types of cells may allow to predict internal concentrations even of quickly biotransformed chemicals accurately.

We219 Recent advances in QSAR prediction of fish and human biotransformation half-lives
E. Papa, Department of Theoretical and Applied Sciences; L. van der Wal, REACH Mastery; J.A. Arnot, ARC Arnot Research & Consulting / Department of Physical Environmental Science; P. Gramatica, University of Insubria / DISTA

The evaluation of biotransformation processes and rates is an important step in exposure and risk assessment procedures for environmental contaminants. Metabolic biotransformation is a key process, which can lower the Bioconcentration Factor of a chemical in fish below what is expected based on its LogKow alone. Biotransformation in humans and other species is also a key process mitigating bioaccumulation and exposures. Unfortunately, the experimental determination of whole-body in vivo metabolic biotransformation rate constants (kB) and half-lives (HL) is expensive and time consuming; therefore, the use of in silico techniques, alternative to animal testing, such as Quantitative Structure Activity Interactions relationships (QSARs), can be applied to predict missing information of new and existing compounds. In this poster, we present recent
advances in the prediction of biotransformation HL in fish and humans by QSAR models. Two data sets with fish and human biotransformation half-lives for over 1000 organic chemicals were used to build QSARs according to the OECD principles for the validation, for regulatory purpose, of (Q)SAR models. Multiple Linear Regression (MLR - Ordinary Least Squares(OLS) method) optimized by Genetic Algorithm Variable Subset Selection procedure, was performed in the software QASARNS using theoretical molecular descriptors calculated from molecular structures. Each compound, employing cryopreserved trout hepatocytes and liver subcellular fractions (S9) can be used to estimate whole-body rates of chemical metabolism. Established in silico models require these rates as inputs to predict chemical bioaccumulation. Predicted levels of accumulation obtained in this manner are generally closer to measured values than predictions obtained assuming no metabolism, suggesting that these methods could provide critical information as part of a tiered approach to bioaccumulation assessment. Regulatory acceptance of these procedures requires, however, that they be evaluated to determine their reproducibility within and among laboratories. Building on previous work, a ring trial is being led by the ILSI Health and Environmental Sciences Institute (HESI) with the aim to support the development of a tiered approach to the prediction of biotransformation. The protocol is not restricted to one specific fish species. Building on this single species extrapolation concept, biotransformation can be calculated while providing a cost-effective assay that uses less vertebrate animals. Building on this single species extrapolation concept, biotransformation data from multiple species may be used to construct a biomagnification or trophic magnification model for a given chemical. Using 14C radio-labelled compounds and high performance liquid radiochromatography, in vitro metabolism data utilizing liver microsomes were developed for a cyclic siloxane, D5, and a linear siloxane, L4. Of the species investigated, minnow demonstrated the greatest potential to biotransform D5 and L4 siloxanes. 14C radiochromatograms show the loss of D5 and L4 siloxane, as well as increases in metabolite production over the 60 min incubation period. The percentage loss of D5 was similar with human and rat microsomes and greater then observed with fish. The percentage loss of D5 in birds is low compared to fish and mammals. Similar relationships for loss of parent hold true for L4 (kestrel data not available). These data suggest that D5 and L4 siloxanes are biotransformed by a wide array of species, which can influence “B” assessments. In addition, these data can be used to estimate whole-body rates of metabolism for incorporation into predictive environmental assessment data where data gaps exist.

WE223

A Critical Review of the Biotransformation of Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5) in Fish

D.B. Huggett, Waterborne Environmental, Inc. / Department of Biological Sciences and Interdisciplinary Science, University of Idaho; E. J. Deetz, Waterborne Environmental, Inc. / Department of Biological Sciences and Interdisciplinary Science, University of Idaho; M.R. Embry, Waterborne Environmental, Inc. / Department of Biological Sciences and Interdisciplinary Science, University of Idaho

Biotransformation of D4 and D5 siloxanes is widely used in consumer products and industrial applications. These two siloxanes have a high octanol-water partition coefficient (log Kow > 6), which is suggestive of a high aqueous bioconcentration factor (BCF). Several studies employing high performance liquid radiochromatography demonstrate that D4 and D5 siloxane are biotransformed into more polar metabolites. A third in vivo study employed whole body autoradiography (WRA) and found that a bulk of the D4 and D5 radioactivity was associated with the liver, gall bladder and digestive tract during and after exposure. In vitro microsomal studies suggest that 14C-D5 was biotransformed by rainbow trout, while minimal biotransformation was observed with common carp and channel catfish. Using these data-sets, an estimated kow for D4 and D5 siloxane is > 0.01 day−1. Based on the available data, there is conclusive evidence that D5 siloxane is biotransformed to more polar metabolites in fish. This biotransformation is important and provides evidence for biodilution behavior in food webs (i.e. a TMF < 1).

WE221

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Metabolism and elimination of 14C-D4 and 14C-D5 in rainbow trout (Oncorhynchus mykiss) J.Y. Domoradzki, R.L. Jeziowsk, Dow Corning Corporation / Health and Environmental Sciences; D.A. McNett, Health and Environmental Sciences; L. Thackery, J.M. Regan, J. Sushinsky, Dow Corning Corporation; J.A. Durham, Dow Corning Corp. / Health and Environmental Sciences; T.A. Springer, T.L. Ross, World Life International Ltd; K.B. Woodburn, Dow Corning Corporation / HES; K.P. Plotzke, Dow Corning / Health Environmental Sciences. Whole-body autoradiography (WBA) was conducted in conjunction with dietary bioaccumulation studies with 14C-D4 and 14C-D5 to assess distribution and metabolism in rainbow trout. For D4, fish were fed 15 mg/kg wet wt (ww) during the uptake phase, livers were collected in the depuration phase and analyzed for parent and total radioactivity. Comparison of the bioaccumativity of D4 to parent concentrations in liver extracts indicated the presence of one or more metabolites. WBA conducted during the depuration phase showed that the highest amounts of radioactivity were present in the digestive tract, liver and adipose tissue, with lesser amounts in other tissues and organs. There was a significant amount of radioactivity in the gall bladder, with moderate amounts in the liver and contents of the digestive tract. Continued presence of parent D4 in the digestive tract contents at 42 days depuration demonstrates elimination of parent D4 via enterohepatic circulation. Similarly, 14C-D4 bioaccumulation study analysis of radioactivity concentration and WBA provide evidence that D4 is metabolized and eliminated via the digestive tract over time. WBA observations during the depuration phase are similar to those found with D4. WBA with metabolism observed in bioaccumulation studies are data (Moore et al. 2001). The bioaccumativity (1.2 mg/mg) of D5 (10.5–1 mg/kg ww) for 4–5 days and whole fish extracts were analyzed for parent and total radioactivity. Percentages of total radioactivity as metabolites were 5% and 31%, for D4 and D5, respectively. Recent in vivo 96-h metabolism studies with rainbow trout confirm the WBA findings. Radioactivity detected in the liver for both D4 and D5 was due to the presence of metabolites (~40%) as a component of the total radioactivity measured. An important aspect observed in these studies was biliary excretion of metabolites (~95% (D4) and 99% (D5)) which is indicative of hepatic metabolism. The km values for D4 (0.15 day−1) and D5 (0.17 day−1) were determined by modeling blood constituents. Consistent with the in vivo findings, is the loss of 14C-D, following incubations with hepatic microsomes from rainbow trout. The work was supported in part by the Global Silicone Industry.

WE225 Suitability of terrestrial isopods for bioaccumulation studies S. Kampe, H. Jürling, Fraunhofer IME Institute for Molecular Biology and Applied Ecology; C. Schlechtinrm, Fraunhofer IME / Okkotoxologie. Physiological differences, i.e. aquatic and terrestrial organisms (lung versus gill ventilation) may result in differences in uptake and depuration kinetics of substances between aquatic and terrestrial organisms. Furthermore, fate and behavior of compounds may be completely different in aquatic and terrestrial systems, depending on their physical-chemical properties. The bioconcentration fish test according to OECD test guideline 305 is the standard test design for bioaccumulation studies (OECD 1997). The bioaccumativity (1.2 mg/mg) of D4 (10.5–1 mg/kg ww) for 4–5 days and whole fish extracts were analyzed for parent and total radioactivity. Percentages of total radioactivity as metabolites were 5% and 31%, for D4 and D5, respectively. Recent in vivo 96-h metabolism studies with rainbow trout confirm the WBA findings. Radioactivity detected in the liver for both D4 and D5 was due to the presence of metabolites (~40%) as a component of the total radioactivity measured. An important aspect observed in these studies was biliary excretion of metabolites (~95% (D4) and 99% (D5)) which is indicative of hepatic metabolism. The km values for D4 (0.15 day−1) and D5 (0.17 day−1) were determined by modeling blood constituents. Consistent with the in vivo findings, is the loss of 14C-D, following incubations with hepatic microsomes from rainbow trout. The work was supported in part by the Global Silicone Industry.

WE226 Bioconcentration tests with fish and the freshwater amphipod Hyallela azteca. Are the results comparable? C. Schlechtinrm, Fraunhofer IME / Okkotoxologie; S. Kampe, H. Buckert, Fraunhofer IME, Institute of Molecular Biology and Applied Ecology; T. Schaefer, Fraunhofer-Institut / Ecotoxicology; J. Lhariorn, LORÉAL / Life Sciences Direction Bioconcentration factors (BCF) for regulatory purposes are usually determined by fish flow-through tests according to TGD OECD 305. Fish bioaccumulation studies are time consuming, expensive, and use many laboratory animals. Alternative methods that replace the use of fish for BCF testing would therefore be of value. The aim of this study was to investigate whether the freshwater amphipod Hyallela azteca can be used as alternative test organism for bioaccumulation studies, providing the opportunity to explain bioaccumulation from water (biocenomination). Nine compounds of different lipophilicity (logKow 3.81–6.92) were tested under flow-through conditions to determine steady-state and kinetic bioconcentration factors (BCFs and BCFk). The following substances were tested: hexachlorobenzene, ortho-terphenyl, benzo(a)pyrene, methyloxochlor, 1,2,3-Trichlorbenzol, 2,4,5-Trichlorphenol, PCB 153, PCB 77; DB[a]anthracene. The results were compared with fish BCF estimates for the same compounds described in the literature. We could show that any of the compounds tested have not potential for bioaccumulation while being bioaccumulated in fish, i.e. no “false negative” prediction of bioaccumulation in fish. This means that the H. azteca bioaccumulation test could be, which needs to be confirmed by further testing, an appropriate test to predict no significant (BCF < 500) and/or no B-BV classification (BCF < 2000) in the standard fish test. Bioaccumulation studies with H. azteca support laboratory animal welfare considerations using a non-vertebrate species, improve efficiency and reduce costs for BCF-testing.

WE227 Development and application of sediment bioaccumulation tests for freshwater benthic invertebrates L.A. Sidney; N. Diepzen, Wageningen University / Department of Aquatic Ecology and Water Quality Management; X. Guo, Peking University / College of urban and environmental science; A.A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group The variability in bioaccumulation and the relative importance of uptake routes of chemicals across benthic species with different traits is insufficiently understood. We measured and modelled bioaccumulation of PCBs for Chromonous riparius (Arthropoda, Hyalella azteca (Crustacea), Lumbriculus variegatus (Annelida) and Sphairum corneum (Mollusca)). We deployed a novel battery test procedure using multiple enclosures in one aquarium, which maximized equality of exposure for the four species, such that the remaining variability was due mostly to biological traits. The relative importance of uptake from either pore water or sediment ingestion was manipulated by using 28 d spiked standard OECD sediment with low (1%) and medium (5%) OM content and one year spiked (aged) sediment with medium OM content. The ranges in the magnitude of biota sediment accumulation factors (BSAF) were C. riparius (3.10 < S. corneum (10–17) < L. variegatus (7–61) < H. azteca (5–114). The high and highly variable BSAF values challenge the presumed value of 1-2 typically employed in ecological risk assessment schemes, and may limit the adequacy of addressing exposure from the pore water only. We argue that enclosure-based battery tests and mechanistic BSAF models improve the quality of the exposure assessment in whole sediment toxicity tests.

WE228 A toxicokinetic study of specifically acting and reactive organic chemicals for the prediction of internal effect concentrations in Scenedesmus vacuolatus C. Voes, Department Bioanalytical Ecotoxicology; A. Kühnert; C. Hugel, Helmholtz Centre for Environmental Research / Effect-Directed Analysis; E. Kuster, Helmholtz Centre for Environmental Research (UFZ) / Department of Bioanalytical Ecotoxicology. The acute toxicity of chemicals is determined well by using the internal effect concentration accounting for differences in toxicokinetic processes and mechanisms of toxic action. This present study examines toxicokinetics of specifically acting and reactive chemicals in the green alga Scenedesmus vacuolatus by using an indirect method. Concentration depletion in the exposure medium was measured for chemicals of lower (log Kow < 3): isoproturon, metazachlor, paraquat and moderate (log Kow 3–5): irgarol, triclofen, N-phenyl-2-naphtylamine) hydrophobicity at seven time points over 240 min or 360 min. Uptake and overall elimination rates were estimated by fitting a toxicokinetic model to the observed concentration depletions. The equilibrium of exposure concentrations was reached within minutes to hours or was even not observed within the exposure period (e.g. ibuprofen). The toxicokinetics of bioaccumulation cannot be explained by the chemical’s hydrophobicity only, but influencing factors such as ionization of chemicals, an ion-trapping mechanism or the potential susceptibility for biotransformation are discussed. Internal effect concentrations associated to 50% inhibition of Scenedesmus vacuolatus reproduction were predicted by linking the bioconcentration kinetics to the effect concentrations which ranged from 0.048 to 0.0086 mg/1 for specifically acting and reactive chemicals. Knowing the time-course of the internal effect concentration may help to understand toxicity processes like delayed toxicity, carry-over toxicity or mixture toxicity in future studies. Keywords: Internal dose; Bioconcentration factor; Critical body burden; Toxicokinetic-toxicodynamic modeling
WE230
Exposure characterization of pesticides and primary metabolites in ground water and human sweat
C. Sweeney, Dalhousie University / Community Health and Epidemiology; J. Kim, Dalhousie University / Community Health Epidemiology; C. Ryan, University of Calgary / Geoscience and Bsc Environmental Science Program; J. Guernsey, Dalhousie University / Department of Community Health and Epidemiology
PEI produces over 30 percent of Canada’s total potato production annually. Correspondingly, pesticide use in PEI is substantial, with as many as 20 different pesticides applied to potato crops each season. Several of these pesticides are classified as “probable human carcinogens” by the U.S. Environmental Protection Agency. Pesticides can leach into the highly permeable, fractured sandstone aquifer, contaminating the sole source of drinking water for PEI residents. Although assumed to be minimal, the degree of exposure to pesticides in PEI ground water is largely unknown. Moreover, the quantitative characterization of pesticide disposition in the body remains unknown. It is hypothesized that considerable amounts of pesticides and their metabolites are excreted in sweat of exposed individuals. The primary objective of this study is to characterize exposure to pesticides and their degradation products/metabolites in ground water, air, soil and exposed individuals in PEI. To gain further insight into the exposure to and characterization of pesticides in the environment and human body, fate and transport of parent compounds and their breakdown products will be examined in PEI environmental samples. The role of eccrine sweat glands in ridding the body of bioaccumulated pesticides will also be investigated through identification and quantitation of pesticides and key metabolites in sweat of exposed individuals; pesticide concentrations in sweat will be compared to those in urine and blood samples of participants. This work will have significant implications for establishing an effective and feasible course of action to reduce or eliminate unacceptable risks associated with environmental pesticide exposure. Future investigations of pesticide bioaccumulation may involve analyses of other biological samples, such as breast milk, hair and nails. Regular sauna therapy could subsequently serve as an effective means of eliminating deleterious pesticides that may otherwise accumulate in the body over time and may ultimately lead to the development of cancers.

WE231
A comparative study of bioaccumulated pesticide and metal levels in fish from two polluted freshwater dams in South Africa utilised by subsistence, recreational and commercial fishermen.
I. Barnhoorn, Zoology; J.C. Van Dyk, University of Johannesburg; I. Wagenaar, University of Johannesburg / Zoology
During recent years, pollution has become an important source of food and especially as a protein source for communities staying in the vicinity of dams with unrestricted access to water. Most of these are subsistence fishermen providing for their families and sometimes they sell fish as a source of income. Between 2005 and 2010 four scientific papers were published on the pesticide and metal concentrations in muscle tissue of the Mozambique Tilapia, Oreochromis mossambicus, and striped cichlid, Ctenopharyngodon idella, from the Albasini Dam, the Roodeplaat Dam (RDPD). Studies regarding organic and inorganic pollutants in fish muscle since then are non-existent or for RDPD limited. During the most recent study at the AD we found that muscle tissue screened positive for a plant growth regulator; DIPN, a N,N-Dimethylalkylamine; Hallcomid M-8-10; 4,4-Bipyridine (Parquat precursor) and an organochlorine; methoxychlor metabolite. This finding is expected because the AD is situated in an area where agricultural and municipal activities are common in the tributaries feeding the dam. Surprisingly the muscle of fish from the RDPD, situated close to an urban area contained traces of the pesticides lidane, aldrin and dieldrin as reported from a previous study. The results from the current study showed water samples from RDPD had concentrations of DDT and metabolites, chlorpyrifos, cis-chlordane, trans-chlordane, dieldrin and aldrin for example. It can therefore be expected that there could be bioaccumulated levels of the same contaminants in fish tissue. Previous human risk analyses (HRA) at both AD and RDPD were a first off and it was recommended that follow-up HRA’s be conducted to verify these findings. The results will also allow for the establishment of guidelines for safe fish consumption by humans as well as regulation of harvesting intensity at AD and RDPD.

WE232
Magnetic properties of the lichen Pseudoverna furfuracea transplanted near a cement plant in NE Italy
D. Kodnik, University of Trieste / Department of Life Sciences; A. Winkel, Naples University / Zoology; M. Fondano Carmeli, University of Innsbruck / Institute of Botany; M. Tretiach, University of Trieste / Department of Life Sciences
The magnetic properties of transplanted samples of the epiphytic lichen Pseudoverna furfuracea (L.) Zopf have been analyzed in the framework of a biomonitoring study in NE Italy focused on a cement plant. The lichen transplants were exposed for 2 months in 40 sites distributed all around the cement plant: 37 sites were located at the knots of a 700 m² test grid covering agricultural, forest and urban areas and a large industrial zone, and 3 sites were located in nearby urban centers. The samples were analyzed for their elemental content by ICP-MS after a mineralization procedure, and the main magnetic parameters were measured with a AGICO MFK1 susceptibility meter coupled to a CS-3 furnace and a Princeton Micromagn Magnetometer in VSM or AGM operating mode. The elemental analysis of the exposed samples revealed a limited impact of the cement plant on the territory, while that of the industrial zone seemed to be generally stronger. The elemental concentration dataset was in substantial agreement with that of the magnetic properties. The cement plant did not have a significant impact on the magnetic properties of the lichens transplanted in the surrounding areas as well. The samples from the industrial area had the highest values of magnetic susceptibility and of saturation/remanent magnetization. The magnetic mineralogy was reasonably uniform throughout the whole set of samples, and was dominated by magnetite in a broad grain-size range. The magnetic mineralogy of the sample nearest to the cement plant was not distinguishable from that of the other lichen samples and did not seem to be clearly linked to the cement to be present in the dusts entrapped by the filter bags of the bag-type dust collector placed inside the chimney above the furnace of the cement plant. The implications of the magnetic characterization of samples on the interpretation of distributional patterns of the air-born particulate matter rich in metals are thoroughly discussed.

Challenges in Wastewater Treatment and Reuse and the Agricultural Use of Manures and Biosolids (P)

WE233
Qualitative and quantitative determination of diclofenac and other pharmaceuticals compounds in the WWTPs of Alcantarilla and Mula (Murcia).
C. Fernández-López, UCAM; J. Guillel, J. Padilla, G. Caravaca, UCAM Catholic University of Murcia; A. Lahora, ESAMUR Regional Entity for Sanitation and Wastewater Treatment in Murcia Region; J. Parsons, University of Amsterdam / IBED
The presence of certain pharmaceuticals in grounds and surface waters is a serious environmental problem as these compounds are biologically active and could affect non-targeted and potentially susceptible species. The occurrence of pharmaceuticals in the environment indicates incomplete removal of these drugs from municipal wastewater treatment plants (WWTPs). The first objective of this proposal is to identify and quantify diclofenac and other pharmaceuticals compounds in the WWTPs of Alcantarilla and Mula (Murcia). The second objective is to evaluate the relative efficiency of different technologies in eliminating diclofenac and other pharmaceuticals compounds and finally to verify if the aggregated concentrations of pharmaceuticals in the studied WWTPs could be extrapolated from commercial sales and consumption data. Influent and effluents wastewater in the WWTPs were sampled over one week (5 days consecutive) of February. Pooled samples were collected over a period of 24 hours in automated samplers. Compound concentrations were quantitatively determined with HPLC-DAD. WWTPs of Alcantarilla works with Activated sludge – Double stage, Lamination, Coagulation, Flocculation, Sand filters (SF) and Ultraviolet disinfection (UV) while WWTPs of Mula works with Activated sludge – Extended aeration, Lamination, Coagulation, Flocculation, Disc filters and Ultraviolet disinfection (UV).

WE234
Screening treated wastewater for pharmaceutical emerging contaminants under high temperature and saline conditions
D. Naghavi Alagoud, D. Tewolde, J. Rodriguez, Masdar Institute / iWater; F. Ahmad
United Arab Emirates (UAE) is one of the world’s most highly water stressed countries. Currently, major cities in the UAE depend on energy-intensive desalination technology for water production, which results in a significant
environmental footprint. At present, treated wastewater meets less than 10% of Abu Dhabi city’s total water demand indicating a substantial room for improvement with respect to water reuse. We have begun work leading to the sustainable and safe reuse of the treated wastewater in Abu Dhabi, UAE. The recycling of treated wastewater, especially for possible unrestricted end-use, demands a thorough characterization of baseline water quality leading to comprehensive human health and environmental risk assessments for different end-use scenarios. As a first step of this effort, we are screening treated wastewater for emerging contaminants such as pharmaceutical compounds. Initial screening is being conducted using time-weighted average sampling followed by UPLC-MS/MS and derivatization-GC-MS. In this paper, we report the occurrence of pharmaceutical micropollutants or emerging contaminants in the influent, effluent and sludge samples. We also compare possible differences in temperature and salinity conditions in UAE on the occurrence of these chemicals by comparing to reported occurrence in colder regions of the world. Keywords: Emerging contaminants, treated wastewater reuse, screening, UPLC-MS/MS

WE235 Seasonal changes in the removal efficiencies and concentrations of pharmaceuticals and personal care products (PPCPs) in wastewater and receiving soils (Jerez de la Frontera, SW Spain) A. Sippel, J.E. Yanez Hera, M. Bornea, M. Boitard, E. González, O. C. B. D. K. Pazdro, University of Tuebingen / Environmental Analysis; P. Stepnowski, University of Gdansk / Faculty of Chemistry in the course of last decade pharmaceuticals have been recognized as relevant environmental contaminants. Special attention should be paid to antibiotic residues. Their continuous input to the environment may strongly affect bacterial populations and induce biological effects in nontarget organisms, potentially disrupting ecosystem processes. seas can be seen as the final sink of the most persistent antibiotic residues, however the availability of data on antibiotic concentrations in marine environment is still limited. This is particularly true for the Baltic Sea, the sea with large, highly populated catchment area, especially in its southern part. Sediment and water samples were collected during r/v “Oceanis” cruises in 2010-2014 period from various locations in the southern Baltic Sea along the polcoast. More than 120 samples were analysed for presence of sulfonamides, trimethoprim, quinolones and tetracyclines. The procedures for the analysis of target compounds involved solid-liquid extraction, SPE disks and tandem SPE technique for separation, enrichment and clean-up and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with electrospray ionization for final quantitative and qualitative analysis. The validation parameters of the developed procedures showed good precision and accuracy with method limits of quantification ranging from 0.29 to 3.71 ng g⁻¹ d.w. sediment, from 0.5 to 10 ng L⁻¹. 14 compounds were identified in the samples at concentration levels of a few to tens ng g⁻¹ d.w. sediment and of a few to tens ng L⁻¹. The occurrence frequency differed significantly among the compounds. The most frequent compounds were: trimethoprim, sulfamethoxazole, oxotetracycline, enrofloxacin and sulfachloropyridazine. In general, these compounds revealed also the highest concentrations. Occurrence of the identified antibiotics was characterized by high spatial and temporal variability. The results indicate contamination of polcoastal waters with antibiotic residues. Consequently, the studies should be continued to establish more extensive chemical database on antibiotics and their degradation products in the Baltic Sea area. More detailed ecotoxicological studies should be also developed to assess more accurately ecological risks related to the presence of antibiotics residues in the Baltic ecosystem.

WE238 Diazinon and cypermethrin concentrations in wastewater and sludge samples V. Jones; M. Gardner, Atkins Ltd; B. Ellor, UKWIR Diacim in and cypermethrin are key components of products used by farmers to control pests on sheep (a process known as ‘sheep dipping’). The European Commission Water Framework Directive and Priority Substances Directive set stringent environmental quality standards (EQS) for diazinon and cypermethrin concentrations, due to their high toxicity to aquatic organisms. As part of a wider programme of monitoring (the Chemical Investigations Programme), measurements of cimarin in and diazinon were taken in water (influent, effluent and throughout the treatment process) and sludge samples from seven wastewater treatment works (WwTWs) across the UK. Despite high removal between influent and final effluent, cypermethrin concentrations in water samples from all works were several orders of magnitude higher than the relevant EQS. This suggests a significant risk of non-compliance with the cypermethrin EQS in receiving waters. Diazinon removal between influent and final effluent was lower than that for cypermethrin, while diazinon concentrations in final effluent samples were always below the relevant EQS. Cypermethrin and diazinon concentrations in water samples were significantly higher at one of the sampling sites compared to all other works sampled; this was a site known to receive inputs from the wool textiles industry. Concentrations of cimarin in and diazinon in sludge were below the limit of detection (0.1 μg g⁻¹) in the vast majority of samples collected in all WwTWs apart from the site affected by wool textile inputs. The occurrence of antibiotics residues in the polcoast marine environment has become a recent research topic. Non-steroidal anti-inflammatory drugs are weak acids acting by reversible or OH-radical mediated reactions at the diamond electrode. 11 of these TPs have been also found in WTP effluents and in microcosms inoculated with microorganisms from a wastewater impacted river sediment. Only two TPs of a WTP and the microcosms didn’t occur in the EC experiments. 5 TPs have been found in surface waters downstream of a WTP. The results reveal degradation pathways which can be easily transferred to other betablockers (e.g. bisoprolol, atenolol) and that EC-LC-MS is a suitable approach to detect new metabolites and transformation products in environmental samples.

WE237 Antibiotic residues in the polcoastal zone - conclusions from 5 years study K. Pazdro, g. siedlewicz, IO PAN / Marine Chemistry and Biochemistry Department; A. Sippel, M. Bornea, M. Boitard, E. González, O. C. B. D. K. Pazdro, University of Tuebingen / Geosciences in the polcoastal zone - conclusions from 5 years study K. Pazdro, g. siedlewicz, IO PAN / Marine Chemistry and Biochemistry Department; A. Sippel, M. Bornea, M. Boitard, E. González, O. C. B. D. K. Pazdro, University of Tuebingen / Geosciences A. Sippel, J.E. Yanez Hera, M. Bornea, M. Boitard, E. González, University of Cadiz / Department of Physical Chemistry; E. González, University of Cadiz / Department of Physical Chemistry; L. Candela, Polytechnic University of Catalonia; P. Lara, SalarBEX, COTRE and Tonalide, showing average concentrations of 92.9, 75.9, 22.8, 4.0, 2.1, 0.2 μg L⁻¹, respectively. Some target PPCPs such as ketoprofen, diclofenac, nitro musks and galaxolide also showed removal efficiencies that were lower than 10%. Additionally, we carried out several sampling campaigns to characterize concentrations in influent wastewater samples from a sewage treatment plant (STP) at Jerez de la Frontera (SW Spain) over a period of 1 year. Overall, simultaneous analysis of 84 pharmaceuticals and 118 PPCPs (and some regulated contaminants such as pesticides or polycyclic aromatic hydrocarbons) was performed by means of two methods relying on solid-phase extraction (SPE) - liquid chromatography/mass spectrometry (LC/MS) and stir bar sorptive extraction (SBSE) - gas chromatography/mass spectrometry (GC/MS), respectively. Most target compounds (>60%) were frequently detected in influent wastewater samples. The highest concentrations were detected in winter and autumn, probably due to lowest biodegradation rates at lowest temperatures. In spite of the Jerez de la Frontera STP showing high removal efficiencies for most targeted TPs, their removal efficiencies and concentrations of Metoprolol in the environment and in water treatment is not well used in the treatment of diseases of the cardiovascular system (e.g. hypertension). Their continuous input to the environment may strongly affect bacterial populations and induce biological effects in nontarget organisms, potentially disrupting ecosystem processes. seas can be seen as the final sink of the most persistent antibiotic residues, however the availability of data on antibiotic concentrations in marine environment is still limited. This is particularly true for the Baltic Sea, the sea with large, highly populated catchment area, especially in its southern part. Sediment and water samples were collected during the ‘Oceanis’ cruises in 2010-2014 period from various locations in the southern Baltic Sea along the polcoast. More than 120 samples were analysed for presence of sulfonamides, trimethoprim, quinolones and tetracyclines. The procedures for the analysis of target compounds involved solid-liquid extraction, SPE disks and tandem SPE technique for isolation, enrichment and clean-up and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with electrospray ionization for final quantitative and qualitative analysis. The validation parameters of the developed procedures showed good precision and accuracy with method limits of quantification ranging from 0.29 to 3.71 ng g⁻¹ d.w. sediment (and from 0.5 to 10 ng L⁻¹. 14 compounds were identified in the samples at concentration levels of a few to tens ng g⁻¹ d.w. sediment and of a few to tens ng L⁻¹. The occurrence frequency differed significantly among the compounds. The most frequent compounds were: trimethoprim, sulfamethoxazole, oxotetracycline, enrofloxacin and sulfachloropyridazine. In general, these compounds revealed also the highest concentrations. Occurrence of the identified antibiotics was characterized by high spatial and temporal variability. The results indicate contamination of polcoastal waters with antibiotic residues. Consequently, the studies should be continued to establish more extensive chemical database on antibiotics and their degradation products in the Baltic Sea area. More detailed ecotoxicological studies should be also developed to assess more accurately ecological risks related to the presence of antibiotics residues in the Baltic ecosystem.

WE239 Antibiotic residues in the Baltic Sea and soil samples from the area where effluent wastewater from the STP is used for irrigation. Many of the same compounds often detected in influent effluent samples detected at concentrations between <1 and 25 ng g⁻¹ in these soil samples. WE236 Metoprolol Metabolites in Microcosm Experiments, Wastewater and Surface Water A. Sippel, J.E. Yanez Hera, University of Tuebingen / Environmental Analytical Chemistry Center for Applied Geosciences; C. Zwiener, Environmental Analytical Chemistry, Center for Applied Geoscience, University of Tuebingen / Geosciences Metoprolol is a frequently prescribed drug of the therapeutic class of betablockers used in the treatment of diseases of the cardiovascular system (e.g. hypertension). Its occurrence in wastewater treatment plant effluents and surface waters and its ecotoxicological significance to water ecosystems and receiving soils is still limited. This is particularly true for the Baltic Sea, the sea with large, highly populated catchment area, especially in its southern part. Sediment and water samples were collected during the ‘Oceanis’ cruises in 2010-2014 period from various locations in the southern Baltic Sea along the polcoast. More than 120 samples were analysed for presence of sulfonamides, trimethoprim, quinolones and tetracyclines. The procedures for the analysis of target compounds involved solid-liquid extraction, SPE disks and tandem SPE technique for isolation, enrichment and clean-up and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with electrospray ionization for final quantitative and qualitative analysis. The validation parameters of the developed procedures showed good precision and accuracy with method limits of quantification ranging from 0.29 to 3.71 ng g⁻¹ d.w. sediment (and from 0.5 to 10 ng L⁻¹. 14 compounds were identified in the samples at concentration levels of a few to tens ng g⁻¹ d.w. sediment and of a few to tens ng L⁻¹. The occurrence frequency differed significantly among the compounds. The most frequent compounds were: trimethoprim, sulfamethoxazole, oxotetracycline, enrofloxacin and sulfachloropyridazine. In general, these compounds revealed also the highest concentrations. Occurrence of the identified antibiotics was characterized by high spatial and temporal variability. The results indicate contamination of polcoastal waters with antibiotic residues. Consequently, the studies should be continued to establish more extensive chemical database on antibiotics and their degradation products in the Baltic Sea area. More detailed ecotoxicological studies should be also developed to assess more accurately ecological risks related to the presence of antibiotics residues in the Baltic ecosystem.
irreversible inhibition of one or both isoforms of the cyclooxygenase enzymes, COX-1 and COX-2, involved in the synthesis of different prostaglandins from arachidonic acid. Acetaminophen, aspirin, and ibuprofen are the most commonly used NSAIDs in Argentina (20%, 28%, 50% respectively). Generally, a small portion (1 to 10%) of the ingested are excreted unaltered in urine but some of them could vary from (28 to 60%). In consequence and due to the lack of sewage treatment most of these NSAIDs are expected to be present in the freshwater of the Rio de la Plata basin in significant quantities. We analyzed 3000 surveys that were conducted in streets which included information about 5 age groups evenly distributed (X ± SD : 20.0 ± 1.57) whom were asked about the use of pharmaceuticals in order to relieve general (17.4%), oesto & joints pains (11.2%) or fever (10.3%) in a period of 30 days before the survey (% answered positively); 28% declared their use. None positive said that they had used at least one NSAID in the period and 4.6% used two different classes and 0.4% used three. An estimation of the annual contribution in Metropolitan Area of Buenos Aires is presented. An extrapolation was perform considering the area served with cloacae sewage, the population size connected to the sewage system, the age pyramid, the average use of each NSAIDs during one month, the diary intake according average weight in each group and duration of treatment, the rate of degradation and the no metabolized excretion rates. Each estimated burden entering annually from cloaca to the river is listed as follows: ibuprofen 1958, aspirin 1388, acetaminophen 656, dipirona 36.0, tramadol 8.28, diclofenac 4.19, ketorolac 0.83, piroxicam 0.62, meloxicam 0.28 and naproxen 0.28 kg/year. The presence of some of these compounds were found solved in the water. Many non-target river organisms comprises a comprehensive survey of 12 PFAAs, which includes 9 PFCAs and 3 PFSAs in environmental waters (STP influent, STP effluent, surface water) collected at three different locations. Ecotoxicity was assessed using standard acute and chronic bioassays. The model substances exhibited high phytoxicity in water, while their effect concentrations covered a broader and more lipophilic residues sorb to the particulate phase (sewage sludge). Thus, when many of these constituents are only partly degraded during wastewater treatment, their more hydrophobic residues remain in the water phase (effluent), whereas their more lipophilic residues sorb to the particulate phase (sewage sludge). Thus, when reusing treated wastewater and sewage sludge in agricultural and landscape management, associated micropollutants may enter and potentially deteriorate the receiving environment. A shortcoming today is that these risks are addressed separately by different legal frameworks, for a limited number of contaminants and isolated for each ecosystem. The present work aimed at assessing the integrated effects of reusing treated wastewater and sewage sludge on land to the environment by chronic and acute bioassays were performed. Samples from semi-field experiments and from prolonged field-scale studies were tested for their ecotoxicological effects on representative organisms. As model substances, three azoles and a quaternary ammonium compound were selected, representing different routes of consumption while also differing in behaviour during wastewater treatment. Toxicity was assessed using standard acute and chronic aquatic and terrestrial bioassays. The model substances exhibited high phytoxicity in water, while their effect concentrations covered a broader and differently ranked range in soil. Effects were not significantly reduced by sludge-amendment to soil. While no risks would be expected from realistic sludge or wastewater application rates, a deterioration of the terrestrial habitat quality cannot be excluded on the long-term. Semi-field experiments on the reuse of treated wastewater supported evidence that soil passage partially improves the habitat quality of wastewater but that this may come at the cost of deteriorating habitat quality of some soils and for some organisms. Thus, it should be carefully decided under which conditions regarding land use and soil properties the benefits from reusing treated wastewater and sewage sludge outweigh the possible deterioration of soil quality. Overall, sewage sludge and treated wastewater constituents may affect both compartments, water and soil, and should therefore be considered in an integrative instead of isolated way.

WE243 Efficient Assessing the Biological Activity of Treated and Untreated Water Using in vitro Bioassays
A.R. Petosa, McGill University / Chemical Engineering; R. Benoit, Chemical Engineering; M. Marshall, McGill University / Chemical Engineering; L. Taylor, McGill University; V.V. Yargeau, McGill University / Chemical Engineering Municipal sewage treatment plants (STPs) in North America’s Great Lakes Basin are point sources for a broad assortment of potential toxins. The inability of STPs to efficiently remove anthropogenic materials (e.g., pesticides, pharmaceuticals, personal care product ingredients) may have unforeseen health impacts and harm aquatic organisms and ecosystems. Given the vast array of chemicals present in municipal wastewater, regulating discharges based solely on compound specific chemical analyses may not be sufficient to ensure STP discharge is free of unwanted contaminants. While the removal of specific indicator compounds can be determined with chemical analyses, the use of in vitro bioassays enables the toxicity of aquatic matrices containing known and unknown materials to be assessed. Herein, cell-based in vitro bioassays were employed to determine whether environmental waters (STP influent, STP effluent, surface water) collected at three STPs in Ontario, Canada’s Great Lakes Basin potentially pose health risks. All
samples underwent solid phase extraction and the extracts were then tested with various bioassays including (i) the CellTitre 96® AQueous One Solution Cell Proliferation Assay (i.e., MTS assay, Promega), (ii) the NR2F Luciferase luminescence assay to monitor cellular antioxidant response, (iii) the EReq CALUX® estrogen receptor assay (Biodetection Systems B.V., Amsterdam), and (iv) the Nuclear Receptors 10-Pathway Reporter Luciferase Kit (Quagen) to investigate multiple pathway regulation in an MCF7 cell line. Indicator compounds responing to a baiting condition were identified by green chemistry tests in environmental samples were also selected and their presence and removal at the STPs was assessed. Outright cell death was found to be an uncommon occurrence. However, it is evident that exposure to anthropogenic compounds in the extracts impacts the different cell lines, resulting in varying levels of reporter gene expression. Furthermore, chemical analyses demonstrate that the presence of indicator compounds and compound removal efficiencies differ for the three STPs investigated. The findings highlight the importance of employing multiple bioassays to investigate specific and non-specific cytotoxic action, and cell pathway regulation. Overall, combining bioassays with a thorough chemical analysis provides a more complete picture of STP effectiveness in removing emerging contaminants.

WE244
Combination of standardized ecotoxicological tests and in situ measurements to characterize the effects of an effluent from a biorefinery on a freshwater ecosystem.
A. Bado-Núñez, UMRI SEBIO; N. Manier, P. Pandard, INERIS; C. Len, UTC ESCOM; A. Gefbard, Université de Reims Champagne Ardenne / Interactions Animal-Environnement (IAE); S. Betoulle, Université de Reims; M. Bonnard, Ecotoxicological Laboratory; J. Porcher, INERIS / UMRI SEBIO
The chemical industry is a very important economic sector in France with used especially oil and their derivates. Nevertheless, the growing concern of economic waste and pollution contributed to the development of green chemistry notably based on utilization of the biomass. The development of this new type of industry is mediated through the generation of bio-refineries. These bio-refineries are able to transform the agricultural and forest production to bio-energy and bio-product. The present work aims to better understand the ecological and environmental impact of green chemistry on freshwater ecosystem. In this context, some fishes and shellfishes were caged during 21 days upstream and downstream a bio-refinary of derived glycerin in order to identify potential immunotoxic and genotoxic effects. In the same way, the environmental impact of bio-refinary effluent was characterized in a regulatory context using standardized ecotoxicity tests (e.g. mobility of Daphnia magna, growth of the unicellular green algae Pseudokirchneriella subcapitata, reproduction of Ceridaphnia dubia). The results of this combined approach will be discussed in the poster.

WE245
Whole Toxicty Assessment of Industrial and Sewage Effluent in Japan
T. Kusui, Toyama Prefectural University / Faculty of Engineering; Y. Iatsu, Toyama Prefectural University / Faculty of Engineering; J. Jun, Toyama Prefectural University / Faculty of Engineering
To manage the effluent quality, whole effluent toxicity (WET) testing has been introduced in many countries such as USA, Canada, European countries and South Korea. The feasibility of introducing the WET approach into the present regulatory framework in Japan has been under discussion although the draft guidelines for WET testing was published in March, 2013. To grasp the ecotoxic potential of the present effluents in Japan, five industrial and four sewage effluents in Toyama Prefecture, Japan were subjected to three freshwater short-term assays (i) the Nuclear Receptors 10-Pathway Reporter Luciferase Kit (Quagen) to investigate multiple pathway regulation in an MCF7 cell line. Indicator compounds responding to a baiting condition were identified by green chemistry tests in environmental samples were also selected and their presence and removal at the STPs was assessed. Outright cell death was found to be an uncommon occurrence. However, it is evident that exposure to anthropogenic compounds in the extracts impacts the different cell lines, resulting in varying levels of reporter gene expression. Furthermore, chemical analyses demonstrate that the presence of indicator compounds and compound removal efficiencies differ for the three STPs investigated. The findings highlight the importance of employing multiple bioassays to investigate specific and non-specific cytotoxic action, and cell pathway regulation. Overall, combining bioassays with a thorough chemical analysis provides a more complete picture of STP effectiveness in removing emerging contaminants.

WE246
Application of a tertiary treatment: Photobiotreatment of WWTP effluents before and after the application of a detoxification treatment process:
M. Martin Garrido, University of Cádiz / Center for Marine Science and Technology CACYTMAR / Chemical Physical Water effluentst are widely recognized as a major source of environmental contaminants. Most wastewaters are processed by municipal wastewater treatment plants (WWTPs). Despite the legal requirement for WWTPs, effluents are still considered important sources of pollution for both marine and freshwater ecosystems. Then sewage effluents are complex mixtures that are known to compromise the health condition of aquatic organisms. Moreover, the environmental repercussions of the discharge of disinfected effluents are still poorly understood. The aim of this study was to determining the environmental risk WWTPs effluents that are discharged in the Bay of Cadiz (SW, Spain) before and after the application of a detoxification treatment process: phytotreatment. The objective of the present study was to compare direct and non-direct approaches to evaluate the toxicity of selected effluents, being the sea urchin larval development and mortality in fish larvae the most sensitive endpoints. The phytotreatment significantly reduced effluents ecotoxicity (p < 0.05). On the other hand, effluents from the different wastewater treatment plants resulted to increase significantly toxicity (p < 0.05) after exposure, since bacteria bioluminescence, survival, growth and reproduction were negatively affected compared with control treatment. Different dilutions of the effluents were analysed in order to propose the incorporation of these short of detoxification treatments which decrease the ecotoxic load and the effluent discharge may cause to the marine aquatic ecosystem of the Bay of Cádiz. Biomarkers of exposure to whole and eulations of two industrial wastes in Vicia faba and Lactuca sativa. C. Dominguez; W.D. Di Marzio, Universidad Nacional de Lujan-CONICET; J. Alberdi, UNLU; M. saenz, Universidad Nacional de Lujan - CONICET
The use of bioassays to evaluate the toxicity of wastes is very important in order to have a more direct and integrated estimate of environmental toxicity. Two industrial wastes, one from foundry sand and the other from cosmetic production were evaluated by means of physico-chemical parameters and exposure biomarkers. Two approaches commonly used in testing of solid wastes may be appropriate for evaluate the ecotoxicological potential of wastes. They are solid phase and water extracts from waste (aqueous elutions). This approach is very important because wear is considered the principal carrier of contamination. The objective of the present study was to compare direct and non-direct approaches using different bioassays and biomarkers in high-risk plants under a global strategy of ecotoxicological assessment of two industrial wastes. Biomass growth, level of lipid peroxidation, and antioxidative stress enzymes (catalase and peroxidase) were assayed in Vicia faba roots for aqueous elutions. Root elongation and seed germination in Lactuca sativa assay were performed for solid waste evaluation. A large range of toxicity responses was obtained with the different endpoint analyzed. Whereas root elongation was a sensitive biomarker for both wastes, lipid peroxidation was not modified regardless of the concentration assayed. The seed germination was inhibited in a dose dependent manner by both wastes. The enzyme activity was inhibited or stimulated according to waste and concentration. The study highlights the importance of performing a battery of bioassays and biomarkers to assess the ecotoxicity of waste before to final disposal. Comparison of the sensitivity of different endpoint analyzed allows choose the most suitable for regulatory purposes.

WE248
Evaluation of hydric resources under influence of a refinery, by ecotoxicological analysis integrated to environmental parameters. M. M. ROBERTO, UNESP - UNIV ESTADUAL PAULISTA / Department of Biology; M. Hoshina, Unesp - Institute of Biology / Biology; R.V. HARA, C.S. ARAUJO, M.P. SILVA, UNESP - UNIV ESTADUAL PAULISTA / Department of Biology; M.A. Marin-Morales, Unesp - Institute of Biology / Biology; D.D. ANGELIS, UNESP - UNIV ESTADUAL PAULISTA / Department of Biochemistry and Microbiology
The petrochemical industry uses large volumes of water and generates big amounts of effluents. Some industries of this sector have been worrying about the surrounding environment quality, attempting to reduce the impact by treating the wastewater, as well as stimulating the biomonitoring. The largest oil refinery in Brazil is located in Paulínia municipality, São Paulo State, between the Jaguari and Atibaia rivers, which supports 10 refineries in the region. This complex wastewater from the Jaguari river, treats the wastewater and discharge it in the Atibaia river. The aim of the present study was: 1) evaluate the cytotoxicity, genotoxicity and mutagenicity of several water samples collected from these rivers through Allium cepa and Oxyreochromis niloticus tests; 2) evaluate those parameters, using the same organisms, on the water samples collected at different stages of wastewater treatment performed by the refinery from 2009 to 2013, and 3) correlate the influence of the physicochemical factors on the endemic biota. The physicochemical analyses were performed and showed that some parameters are in disagreement with current law, affecting the water quality of these rivers. Meteorological data were also registered and showed a correlation with the
recorded cytotoxicity and genotoxicity effects, mainly in the early periods of intense rainfall. The water collected at the refinery collection point and at the upstream of the effluent discharge point have shown worrying toxicity levels, which may be a strong evidence of the influence of other human activities. The meteorological data showed defined hot and rainy seasons (spring and summer) and cooler and drier periods (autumn and winter), which combined to the physicochemical results, were useful for a better comprehension of the biological results. From all the data obtained, a multivariate analysis was performed to determine the main environmental factors that may be directly related to the effects obtained by biological assays. The results of this mathematical method were satisfactory, especially when applied to the data obtained by tests realized with more sensitive organisms, such as fish. From the results of this study we warn that, in spite of the influence of the refinery being small on the biota and the water rivers quality, the monitoring of this area must be constant.

WE249
Cytoxic, genotoxic and mutagenic effects of humic acids from landfill leachate in plants: an integrative approach using multivariate analysis
M. Morezek, UFS Car / Department of Sciences Fisiológicas; M. Bon-Art, Universidade Federal do Espírito Santo / Departamento de Ciências Biológicas; Ld. Souza, Federal University of Sao Carlos / Department of Physiological Sciences; L. Rocha, L. Duarte, Universidade Federal do Espírito Santo / Departamento de Ciências Biológicas; L.B. Dobbs, University of Vila Velha; E.V. Castro, M.W. Lima, Federal University of Espírito Santo; M.N. Fernandes, Universidade Federal do Sao Carlos / Ciências Fisiológicas; S. Matsumoto, Universidade Federal do Espírito Santo

The increasing solid waste production is an important and emergent problem in which landfill dispose represents the most common and appropriate alternative for final disposal. Due to high organic and inorganic pollutant rates present in landfill leachate, proper treatment and disposal methods of this residue becomes extremely important in order to avoid soil and water contamination. Landfill leachate sludge generated after biological treatment is mainly compounded by humic substances (HS) that has influence in plant metabolism. Among the HS, humic acids (HA) are commonly study and presents several stimulatory effects in plants due to liberation of bioactive molecules which can access receptors inside or outside the cell. This study aimed to characterize HA extracted from landfill sludge and assess the effects of different exposure doses in plants through chemical and biological analysis, to elucidate the effects of this organic material and minimize potential risks of using sludge in nature. Multivariate statistical methods were applied to datasets using factor analysis (FA) and Spearman’s rank correlation coefficient. Landfill HA showed high levels of carbon and nitrogen which may represent higher availability of nutrients to plants. ATPases and H+-ATPases increased in plants treated with higher concentrations of HA tested (2 mM C \(^{-1}\)). Additionally, cytogenetic alterations were observed on meristematic and FI cells such as nuclear abnormalities and micronuclei. Multivariate statistical analysis was efficient to integrate data and provide an overview of the physical, chemical and biological results. Thus, despite all numerical data of HS of HA and its activation of plant antioxidant system, the biological effects observed show concern levels of mutagenicity. Due to the residue complexity the use of chemical, genetic and enzymatic analyzers was an important tool for evaluating humic acid application in plants.

WE250
Irrigation with treated wastewater: insights into microbialological implications in soil fertility and in human health
C. Becerra-Castro, CBQF – UCP / CBQF Centro de Biotecnologia e Química Fina; A. Lopes, Faculdade de Engenharia - Universidade do Porto / LEPABE Laboratório de Engenharia de Processos Ambiente Biotecnologia e Energia; I. Vaz-Moreira, Escola Superior de Biotecnologia - Universidade Católica Portuguesa / CBQF Centro de Biotecnologia e Química Fina; E.F. Silva, Instituto Politécnico de Viseu / Escola Superior de Tecnologia e Gestão; C.M. Manaia, CBQF Centro de Biotecnologia e Química Fina; O.C. Nunes, Faculdade de Engenharia - Universidade do Porto / LEPABE Laboratório de Engenharia de Processos Ambiente Biotecnologia e Energia

Agriculture’s increasing dependency on soil and wastewater microbiology gives unequivocal indications that the use of treated wastewater for irrigation, in spite of the unquestionable benefits, may have adverse consequences on soil productivity and fertility, and pose serious risks to the environmental and human health. Information currently available shows that physicochemical alterations, such as the increase of organic matter related pools, salinity and soil accumulation of contaminants are affected with treated wastewater. The soil microbiota, with a key role on its fertility and productivity, is also known to respond to alterations imposed by wastewater irrigation, presenting an increase of microbial biomass and activity and alterations in community structure. In spite of these observations, the available literature shows that the effects on soil microbiota are neglected when the implications of irrigation with wastewater are discussed. In the medium and long term risks for human health posed by wastewater irrigation must also be at the heart of any discussion on wastewater reuse. The evidences reported so far highlight the importance of the accumulation and propagation of biological contaminants in soils due to wastewater irrigation. Indeed, numerous chemical contaminants, such as pharmaceuticals and metals, can threaten the environment and human health. Moreover, the mixture of these contaminants may have unpredictable consequences. Biological contamination by human and animal pathogens, phytopathogens and antibiotic resistant bacteria and their resistance genes must also be taken into account, since once transported by wastewater, they may proliferate and be transmitted over different environmental compartments. Studies on the effects of wastewater irrigation on soil are challenging since direct cause-effect relationships can hardly be found. The use of multi-parametrical analyses and holistic studies may bring additional and reliable insights into this problem. The maintenance of a rich and diversified autochthonous soil microbiota and the use of treated wastewater with minimal levels of potential soil contaminants are proposed as sine qua non conditions to be achieved in soil. It has been shown in other studies that these chemicals can be present in the soil environment after irrigation with wastewater. The aims of this work are to identify the sources, fate and movement of APIs to soils with particular reference to industrial and countries and produce data to evaluate existing risk assessment outcomes and to assist in further developing methodologies. Gaps in understanding and exposure pathways of APIs in industrialising countries have been identified as part of a literature review. Key areas addressed as part of this review are (i) lack of knowledge of the amount and types of APIs being consumed, leading to issues modelling concentrations in wastewater; (ii) API removal efficiencies of sewage treatment plants; (iii) movement of APIs to groundwater and other water bodies and (iv) the uptake of APIs by crop plants in these areas due to possible higher concentrations of APIs in wastewater/sluage. Further work as part of this project will include amendment of soil amended with wastewater and sludge containing priority APIs. The potential outcomes of this research are to provide data for modelling scenarios, to improve ERAs for APIs in soil and to aid understanding of the gaps identified in research.

WE251
Environmental Risk Assessment of Active Pharmaceutical Ingredients in Soils with Emphasis on Wastewater Reuse Practices
K. Lees, Biogeochemistry Research Group; M. Fitzsimons, Plymouth University; J.R. Snape, AstraZeneca UK Ltd / AstraZeneca Global Environment; A. Tappin, Plymouth University; S. Comber, Plymouth University / Environmental Science

This project will address the production and use of active pharmaceutical ingredients (API) as part of a marketing notification in Europe and North America. However, an adverse environmental risk profile is not a barrier to approval or to patient access to medicines. This ERA framework is predominantly focused on assessing the risks that APIs pose to aquatic life. There are currently no requirements for a soil risk assessment to be carried out on APIs which do not strenuously sorb to soils, i.e. those APIs with a log Koc < 4. However, in emerging markets where sufficient infrastructure and/or waste management is lacking this can lead to the direct discharge of poorly treated or untreated wastewater and/or sludge being applied to agricultural fields. The fate of APIs in wastewater and sludge used on soil has not received much attention and their fate is not well understood in soil. It has been shown in other studies that these chemicals can be present in the soil environment after irrigation with wastewater. The aims of this work are to identify the sources, fate and movement of APIs to soils with particular reference to industrialising countries and produce data to evaluate existing risk assessment outcomes and to assist in further developing methodologies. Gaps in understanding and exposure pathways of APIs in industrialising countries have been identified as part of a literature review. Key areas addressed as part of this review are (i) lack of knowledge of the amount and types of APIs being consumed, leading to issues modelling concentrations in wastewater; (ii) API removal efficiencies of sewage treatment plants; (iii) movement of APIs to groundwater and other water bodies and (iv) the uptake of APIs by crop plants in these areas due to possible higher concentrations of APIs in wastewater/sluage. Further work as part of this project will include studies on the in situ fate of APIs in soil amended with wastewater and sludge containing priority APIs. The potential outcomes of this research are to provide data for modelling scenarios, to improve ERAs for APIs in soil and to aid understanding of the gaps identified in research.

WE252
UNCSD Rio+20 FOCAL POINT ON INTERNATIONAL WATER COOPERATION
D.P. Bindra, Civil Aviation / UNCSD Rio Focal Point

The paper aims at contributing to the elaboration of the water-related Sustainable Development Goals and provide concrete guidance on the most pressing Libyan water issues – drinking water, sanitation, waste water treatment, integrated water development & management, international water cooperation, and innovative water technologies needed for manmade river. After giving a background on Agenda 21 adopted at the UN Conference on Environment and Development in Rio de Janeiro, Brazil, in June 1992 the paper focuses on effective ways to make cooperation happen in the water domain. The best approaches to promote effective cooperation will be presented (and to do better in water cooperation. Based on UNCSD Rio+20 Working Group Discussion Topics numerous Strategic Area in the National Action Plan are identified: 1 Enabling environment, 2 Public-private partnerships, 3. Inter-sector coordination 4. Implementation, 5. Advocacy, 6. Integration, 7. Promotion, 8. Monitoring and evaluation and 9 Research. A case on Environment Aspects of the European Neighborhood Policy (ENP): Water Focus is presented to demonstrate that how New Libya can avail ENPI Funding opportunities for Environment/water related focus.
accumulation of pollutants by farmed species. Therefore, mitigation measures and management tools are needed to ensure the development of sustainable aquaculture and to decrease the health-related risks for consumers. In this context modeling may be used as a scientific and technical tool to support the evaluation of water quality and the effectiveness of mitigation measures. In the north-eastern part of Italy, in the Friuli Venezia Giulia Region, the Marano and Grado Lagoon is the most complete coastal lagoon ecosystem with the presence of important socio-economic activities in the lagoon, such as traditional fishing and shellfish farming. Numerical simulations of the Marano and Grado lagoon were performed for hydrodynamic circulation, temperature and salinity behavior with the SHYFEM model, a shallow water finite element model developed at ISMAR-CNR in Venice (Ferrarin et al., 2010) and experimental data were used to calibrate the numerical model. The numerical model SHYFEM may be applied also to support the traditional monitoring methods and connect the information concerning the hydrodynamic circulation with the microbiological parameters. In this study scenarios of bacterial dispersion and climate change events in the Marano and Grado Lagoon are investigated with the SHYFEM model. Many simulations have been performed taking into account different input concentration values from the rivers discharging into the lagoon and different survival times for the bacteria. Events of heavy rain are prescribed as boundary conditions and increased water levels are imposed, too. Results are obtained as concentration time series and maps of concentration, in order to investigate the impact of the bacterial load coming from each river. Results show that, due to particular meteo-marine conditions and in case of high input concentration values, some rivers may influence the water quality in the shellfish farming areas. References Ferrarin C. et al., 2010, Hydraulic zonation of the lagoons of Marano and Grado, Italy. A modelling approach. Estuarine, Coastal and Shelf Science, Vol. 87 (2010), pp. 561-572.

WE254 Impact of treated wastewater discharges on the assessment of antibiotic resistance in the environment

C. DAGOT, ENSIL, P. Marie-Cecile, B. Olivier, Université de Limoges / INSERM, C. Magali, Université de Limoges; G. Margaux, Université de Limoges / INSERM.

The spread of antibiotic-resistant bacteria is a growing problem and a public health issue. Urban Wastewater and Hospital wastewater are considered to be sources for antibiotic resistance dissemination by horizontal gene transfer. Mobile integrons (MI) are genetic elements that acquire, exchange and express antibiotic-resistance genes embedded within gene cassettes (GC). The spread of antibiotic resistance has been monitored on a pilot site in France (http://www.graie.org/Sipibel/). It brings together, in one wastewater treatment plant, effluent from the city (Bellecombe, France) and the hospital (CHUV) discharging in the Seine river. Repetitive GC were conducted on the two types of effluents, upstream and downstream biological treatment facilities, until discharge point in the river (Arve, France). These campaigns measurements were started before the start-up of the hospital activities to have an initial state of the environment. The measured parameters included the classic parameters of pollution, about fifty care activities molecules, assessment of ecosystem health and antibiotic resistance. Measuring the amount of MI directly on the samples and the relative abundance, which is the ratio between the concentration of multiresistance integrons (MRI) and quantification of the gene encoding the 16S rRNA, carries out the evaluation of the global evolution of resistance. The first results showed the presence of antibiotic-resistant bacteria in urban and hospital effluent to similar levels to other treatment plants and hospital effluents. MI class 1 integrating from the hospital are present in similar amounts of the same order of magnitude, even if the hospital effluent seems to be more concentrated in antibiotic-resistant bacteria. These concentrations are also followed in the river from the beginning of the sample campaigns showing the evolution of MRI in the environment affected by the water discharge.

WE255 Fate of antibiotic resistance genes discharged in wastewater: A Microcosm Study

C. Narciso da Rocha, CBQF - UCP / CBQF; C. M. Manaa, CBQF Centro de Biotecnologia e Quimica Maria Helena

To assess the risks associated with antibiotic resistance dissemination from a wastewater source, it would be important to improve our knowledge on the fate of genetic determinants and their hosts, and how this fate can be influenced either by the receiving environment or the bacteria or genetic determinants themselves. These were major objectives of this study. Using microcosm assays from hospital effluent and raw wastewater bacterial community (DGGE analysis) was assessed. After the incubation period at 18 or 30ºC, the number of 16S rRNA and intI1 gene was assessed. After the incubation period at 18 or 30ºC, the number of 16S rRNA and intI1 gene was assessed. After the incubation period at 18 or 30ºC, the number of 16S rRNA gene was assessed by qPCR. The possible influence of the bacterial gene host and of the autochthonous wastewater bacterial community (DGGE analysis) was assessed. After the incubation period at 18 or 30 ºC, the number of 16S rRNA and intI1 gene copy numbers /ng of DNA in sterile water increased or did not vary depending on the gene hosts used (K. pneumoniae or E. coli), intI1 presented a slight increase and vanA decreased irrespective of the gene host. In non-inoculated wastewater microcosm assays, in general, the gene 16S rRNA and intI1 did not vary, while blaTEM and vanA decreased significantly. In inoculated wastewater microcosms the number of 16S rRNA gene copy number /µg of DNA increased when the Enterococcus faecalis and K. pneumoniae were inoculated, but not when Enterococcus faecium and E. coli were used. All the other tested genes harbored by the introduced strains were significantly reduced after 15 days of incubation. No significant correlations were observed over the incubation period, neither at 18ºC nor at 30ºC, for community the richness, diversity and evenness indices. These results suggest that the host of a resistance gene, such as blaaTEM, may influence its fate in the environment. Moreover, it is suggested that genes such as vanA and blaaTEM may decay in the environment, either if they are autochthonous in wastewater or inoculated and irrespective of the gene host. However, even below the detection limit, the methods used do not indicate that these genes were eliminated.

WE256 Conventional PCR as a low cost method to have an overview of the presence of antibiotic resistance genes in wastewater samples

G. Macedo, CBQF Centro de Biotecnologia e Quimica Fina; C. Narciso da Rocha, CBQF - UCP / CBQF; C. M. Manaa, CBQF Centro de Biotecnologia e Quimica Fina

Currently, quantitative real-time PCR (qPCR) is the method of choice to assess the abundance of antibiotic resistance genes (ARG) in environmental samples. Despite the overall reduction of costs, most laboratories handling monitoring analyses may not afford qPCR as routine practice. Therefore, conventional PCR may be considered a good alternative, and the standardization of reliable and easy to perform methods will encourage the routine monitoring of ARG in wastewater samples. Our aim in this work was to ascertain the limit of detection of conventional PCR for some selected indicator genes - class 1 integrase intI1, and blaaTEM and vanA, in wastewater samples with different characteristics, microbial loads, and total DNA concentrations. As a reference method to assess gene abundance was used qPCR. Samples come from hospital effluent, and raw and treated wastewater of the municipal wastewater treatment plant receiving the hospital discharge. Total DNA extracts were obtained from 25 and 150 ml of wastewater respectively, filtered through a 0.22 µm-pore polycarbonate membrane, and using the PowerWater DNA Isolation Kit. Conventional PCR assays were performed in DNA extracts in a range of dilutions of 1-100. Using conventional PCR, the limit of detection of the gene intI1 varied from 1586 to 6 copies/ng DNA (or 5560-203 copies of 16S rRNA gene). For blaaTEM the detection limit varied from 260 to 32 copies/ng DNA (or 12605-5560 copies of 16S rRNA gene). The gene vanA, only detectable in municipal wastewater using qPCR, could be detected in hospital effluent DNA extracts with a detection limit of 340 copies/ng DNA (or 5560 copies of 16S rRNA gene). Sample results suggest that conventional PCR may be a good alternative to qPCR to assess the occurrence of contaminant antibiotic resistance genes in wastewater samples. Critical parameters will be the volume of water to be sampled and the concentration of DNA to test. Acknowledgements: This work was performed under the scope of the project 38900 - NEPCAT financed by FEDER funds through ON2 – Programa Operacional Regional do Norte. Keywords: real-time PCR; conventional PCR; wastewater; antibiotic resistance.

WE257 Quantification of antibiotic resistance genes in wastewater treatment plant and release of resistance genes after the purification process

A. Karkkinen, Department of Food and Environmental Sciences; T.A. Johnson, Michigan State University / Center for Microbial Ecology Department of Civil and Environmental Engineering; M. Tamminen, University of Helsinki; R.D. Stedfield, J.M. Tiedje, Michigan State University / Center for Microbial Ecology Department of Civil and Environmental Engineering; M. Virta, Antibiotic resistant bacteria have become a serious threat to human health and we might be heading to post-antibiotic era. Major concern is the resistance against last resort antibiotics, such as vancomycin, cephalosporins, carbapenems and quinolones. Wastewater treatment plants are known reservoirs for various antibiotic resistance genes and may serve as hotspots for horizontal gene transfer. The resistance genes from the wastewater treatment plants can eventually end up in the environment either in the effluents or in the sludge. Though antibiotic resistance has been studied at wastewater treatment plants, these studies are mainly focused on characterizing more than few genes or studying the fate of the resistance genes after the purification process. In this study we characterized and quantified genes related to antibiotic resistance and horizontal gene transfer in a large-scale wastewater treatment plant and estimated the release of genes to the environment using a parallel qPCR assay targeting 295 genes. All the results were normalized with 16S rRNA gene copy number as the internal standard. These results suggest that conventional antibiotic resistance genes changes in the process and that the diversity and abundance of the resistance genes is lower in the effluent and sludge than in the incoming water. Still many resistance genes are released to the environment and could be detected in sediments near the effluent release site. There was also an increase in the relative abundance of some antibiotic resistance and horizontal transfer genes during the purification process.

WE258 Persistence of resistance genes in sewage canalization networks of a metropolitan area. Relationship between antibiotic selective pressure and
antibiotic resistance genes

S. Caucci, TU Dresden / Institute of Hydrobiology; A. Karkman, Department of Food and Environmental Sciences; M. Virta; T. Berendonk, Institute für Hydrobiologie

The occurrence of antibiotics in natural and urban environments seems to favor the development and spread of antibiotic resistance. At the same time the key factor for the selection of antibiotic resistant bacteria is the ability of an organism to adapt quickly to new environmental conditions. One of the environments where the selective pressure of antibiotic pollution could exert an effect on microbial communities is the urban water system. The sewage canalisations and waste water treatment plants in particular are known to carry elevated concentration of pollutants and microorganisms. The co-existence of these two factors could lead to a gradual increase in the prevalence of resistance within the indigenous microbial community. In our study raw waters originated from the sewage pipes of the main neighborhoods of Dresden metropolitan area (Germany) were seasonally collected along the years 2012-13 and screened for the presence and amount of antibiotic resistance genes (ARG). The genes of interest belonged to the chemical classes of β-lactams, quinolones, macrolides, sulfonamides and tetracyclines. Concentrations of antibiotic residues in the raw waters and effluent were measured and the correlation between the amount of prescribed antibiotics antibiotic residues and antibiotic resistance genes revealed via multivariate statistical analysis. Moreover the diversity and species distribution of microbial communities inhabiting sewage waters and effluent will help us to explain the seasonal variation of ARGs content. Overall, despite the sanitation processes the effluent did not show assistance in inactivation of the ARGs and new wastewater technologies capable to limit the spread of ARGs in freshwater ecosystems.

WE259

Database mining to evaluate antibiotic resistance hotspot risk in the French section of the Mosel and Mosel watersheds

M. PONS, LRGP-CNRS-UL / Laboratoire Réactions et Génie des Procédés; C. Merlin, Laboratoire de Chimie Physique et Microbiologie pour l'Environnement

Antibiotic resistance is a recent concern in terms of micropollution of aquatic environments and the prevalence data in rivers are still scarce. However the availability of data on the presence of indicator microorganisms such as coliforms, enterococci or Es. coli is higher. Concentrations of macropollutants such as ammonium which can be linked to untreated or ill-treated municipal sewage, run-off from pastures or leakage from manure treatment facilities are much better documented as well as those of metals (in liquid phase or adsorbed on sediment) and are available in the Rhin-Meuse Agency Information System (http://rhin-muse.cafrance.fr/). Finally some pharmaceutical residuals are also noted in the database data on the occurrence of pressure (population distribution in the watershed, consumption of pharmaceuticals, discharges from wastewater treatment plants), industrial activities (steel milling, etc.) as well as on agriculture (distribution of cattle units in the watershed, etc.) can be extracted from different database. Various correlations have been tested between the different data to propose a scheme for assessing the risk for hotspot of antibiotic resistance in France. The results will be discussed with respect to available field data concerning antibiotic resistance.

WE260

Antibiotic resistant bacteria in surface waters downstream of farming and waste water treatment sites

M. Laht, Institute of Technology; K. Telling, University of Tartu / Department of Microbiology; V. Kisand, T. Margus, University of Tartu / Institute of Technology; S. Kõljalg, University of Tartu / Department of Microbiology; P. Kalmus, Estonian University of Life Sciences / Institute of Veterinary Medicine and Animal Sciences; I. Lutsar, University of Tartu / Department of Microbiology; T. Tenson, University of Tartu / Institute of Technology

The antibiotic resistant bacteria have been extensively studied in recent years, and their role as a real threat for human and animal health is recognized. We studied the spread of antibiotic resistant bacteria in surface water bodies downstream of agricultural and municipal waste water treatment sites. The sampling program was focused spatially to small areas in Estonia. The cultivated strains were characterized by whole genome sequencing. Samples were collected during years 2012 – 2014 in total from 16 sampling campaignscovering different seasons. Sampling points were related to 4 farms including samples from animals(n=120), slaughter(n=11) and manure(n=10), soil(n=18) from fields and surface water from streams and rivers connected to the farms. In addition, the isolates from city (100 000 inhabitants) wastewater treatment plant were also selected and were selected by DNA sequencing. The analysis was focused on potentially pathogenic bacterial species, such as Escherichia coli (n=166), Klebsiella pneumoniae (n=16), Pseudomonas aeruginosa (n=90) and Staphylococcus aureus (n=27). All isolates were cultivated on commercially available antibiotic containing selective media that are used in medical and veterinary diagnostics for isolation of the species under study. For determining resistance levels of the isolated strains and for comparison with global databases, MLST analysis was used on seven house keeping gene sequences extracted from the whole genome sequences. The resistance patterns were determined for 10 antibiotics specifically recommended for treatment of the infections caused by studied bacteria, using EpiSolver test (E-test). Phenotypic resistance was compared to the betalactamase genes predicted from the genomic sequences. Our studies show that Ps. aeruginosa and E.coli strains can spread from agricultural sites and waste water treatment plants to the downstream environments. The high variety of phenotypic resistance in bacteria of the same sequence type suggests rapid horizontal transfer of resistance.

WE261

Beta-lactamases resistance genes and their genomic localization in environmental bacteria

M. Piotrowska, University of Warsaw, Faculty of Biology, Institute of Microbiology / Department of Applied Microbiology; D. Przygodzińska, A. Krawczyk-Balska, M. Popowska, University of Warsaw / Faculty of Biology, Institute of Hydrobiology and Water Treatment Technology (WTPT) and Technical University of Krakow (TU Krakow)

The evolution of new antibiotic resistances is the ability of an organism to adapt during selection, with example trace amounts of antibiotics or other metal oxides such as copper (Cu) and zinc (Zn) are still widely used as animal growth promoters and may accumulate in agricultural soils receiving animal manures or metal-based pesticides. We hypothesize that Cu and Zn may exert stronger and more persistent selection pressures for development of antibiotic resistance in soil as compared to the antibiotics themselves. We here compare the ability of metals (Cu or Zn) and tetracycline to co-select or to select for tetracycline resistance of a bacterial community in a microcosm study. Metal layered laboratory microcosms were setup by spiking agricultural soil with different levels of Cu, Zn or tetracycline. Pollution-induced community tolerance (PCT) to tetracycline was measured in all microcosms, using the [H]lucene incorporation technique, after 2 months of...
community selection. In situ bacterial growth (1% leucine incorporation), soil pH (pH<6.86) and toxicant bioavailability (whole-cell bacterial bioensors specific for either Cu, Zn or tetracycline) were monitored during the whole community selection phase. Bacterial community tolerance to tetraacycline increased significantly in soils spiked with environmentally relevant levels of Cu (≥333.3 mg/kg) and Zn (≥500 mg/kg), but not in soil spiked with high, realistic levels of tetracycline (≥100 mg/kg). Selection for community-level tetraacycline resistance was correlated to the initial toxicant-induced bacterial growth inhibition. We hypothesize that the low selection pressure posed by tetracycline can be explained by low bioavailability of tetracycline in soil (bioreporter measurements) and not by a bacterial resistance to the antibiotic itself.

WE264 Occurrence of antibiotic resistance in marine sediments and fauna along an exposure gradient in Arctic Greenland

M.E. Granberg, Norwegian Polar Institute / Research; I. Dahlolf, University of Gothenburg / Department of Biological and Environmental Sciences.

The release of antibiotic/antimicrobial substances into the coastal environment may stimulate antibiotic resistance (AR) in marine bacteria. Sewage treatment is generally lacking in Greenland and municipal wastewater is discharged directly into the ocean, likely creating strong gradients of pharmaceuticals from sources into the relatively pristine environment. In accordance with examples from the food industry, we hypothesize that AR bacteria present in the gut of marine organisms may travel from prey to predator along food chains. Greenlandic people traditionally depend heavily on the oceans for food, and our results may thus have implications for the risk of AR exposure to humans. We investigated the correlation between environmental antibiotic exposure and prevalence of AR at three different trophic levels, sediments, blue mussels, fish, along a pollution gradient in Sisimiut, Greenland. During two consecutive summers, sediment, blue mussels (Mytilus edulis) and Arctic sculpin (Myoxocephalus scorpioides) were collected off the town of Sisimiut, Greenland. Bacteria were cultured from animal gut tissues and sediment samples and tested for resistance towards Penicillin, Ampicillin, Amoxicillin and Ciprofloxacin, ranging from natural to semi-synthetic and synthetic antibiotics. AR bacteria from gut tissues were identified with API tests. There was a strong gradient of AR from point sources to more open waters for all antibiotics, but the magnitudes of resistance and the resistance fingerprints differed among sites. There was also a parallel gradient in the controls with respect to the number of colony forming units, indicating a lower bacterial gut content in fish and mussels closer to sewage outlets. AR bacteria from samples collected closer to the sewage outlet were more often identified as human enterobacteria than AR bacteria obtained further from the source. Results indicate the presence of natural AR in pristine areas and a clear human imprint on bacterial AR in the marine environment around Greenland.

WE265 Degradation of cyclophosphamide and ifosfamide by biological treatment and advanced oxidation processes

E. Heath, Jozef Stefan Institute / Env Sciences; M. Cesen, JSI; B. Kompare, University of Ljubljana; T. Kosjek, Jozef Stefan Institute / Department of Environmental Sciences.

The trend towards the increasing use of chemotherapy among cancer patients raises questions concerning the presence of cytostatic drug residues in the environment and the impact they have on ecosystems. In the present study, we report on biological treatment using attached-growth biomass on special carriers and various advanced oxidation processes (AOPs) of two cytostatics: cyclophosphamide (CP) and ifosfamide (IF) in aqueous environment. The average removal during biological treatment using attached growth biomass in a flow-through bioreactor with artificial wastewater were 41.2 % and 18.2 % for CP and IF, respectively. In case of biological treatment of hospital wastewater, the removals were slightly higher, 58.9% and 34.9% for CP and IF, respectively. To achieve higher removal efficiencies, the following advanced oxidation processes were investigated: UV, ozone, UV/ozone, UV/hydrogen peroxide (H₂O₂), ozone/H₂O₂ and UV/ozone/H₂O₂. The efficiency of these conditions was measured regarding the duration of UV and/or ozonation treatment (20 to 120 minutes) and the amount of added H₂O₂ (0 – 5000 mg L⁻¹). The highest removal efficiencies, 98.8 % for CP and 94.0 % for IF, were achieved using UV/O/H₂O₂ at 5 g L⁻¹ for 120 minutes. The same treatment was then applied to hospital wastewater, where similar efficiencies were achieved: CP (98.4 %; IF: 97.9 %). Coupling biological treatment to this AOP (UV/O/H₂O₂) enabled more than 99 % removal of both compounds. To our knowledge this is the first study to report AOP removal efficiencies of cytostatics from non-treated wastewater. Further work will include ecotoxicity testing of investigated compounds and an assessment of treatment efficiency in terms of toxicity. In addition, occurrence of CP and IF metabolites/transformation products in treated samples will be evaluated.

WE266 Reduction of antibiotics & antibiotic resistant Enterococcus in urban wastewater intended for reuse by solar-driven Fenton oxidation


The presence of pathogenic antibiotic-resistant bacteria (ARB) and genes in aquatic environments is an issue of emerging concern, as their incidence has risen due to their continuous exposure to sub-inhibitory concentrations of antibiotics during conventional wastewater (WW) treatment. As urban WW treatment plant effluents are among the main anthropogenic sources of antibiotics into the environment, there is a need of examination of the possible ways of elimination of these mixtures and genes in effluents. In this study, the removal of target antibiotic of 10 µg/L of antibiotics i.e. sulfamethoxazole (SMX) and clarithromycin (CLA) was studied with the use of a HPLC-UV system. In addition, the reduction of Enterococcus populations and the removal potential of those resistant to: a) SMX, b) CLA and c) both antibiotics were examined with the use of selective agar media containing the antibiotics, while the toxicity of the treated effluent was examined with the marine bacterium Vibrio fischeri. Solar Fenton oxidation using natural irradiation with Fenton reagent doses of 50 mg L⁻¹ of H₂O₂ and 5 mg L⁻¹ of Fe⁺ at a pilot-scale was used to examine the disinfection and ARB removal in various aqueous matrices. Solar Fenton was more efficient in the removal of enterococci and antibiotics, compared to photolysis. In detail, in all aqueous matrices SMX had higher removal than CLA (95 % and 70% removal in real WW, respectively). The disinfection of CP and IF was in all inoculated matrices complete after 10 min, 15 min and 60 min of ozonation, the cell counts were below the detection limit (1 CFU/ 100 mL) for distilled water and real WW and after 150 min, 240 minutes for simulated WW. The presence of antibiotic-resistant enterococci exhibited a 5-log reduction with solar Fenton in the real WW effluent in the presence of both antibiotics, separately and in a mixture. After treatment, there were 10 times more antibiotic-resistant enterococci in the presence of SMX than in the presence of CLA. Finally, the toxicity of the solar Fenton remained at low levels throughout the experimental period. Acknowledgements: The experimental work was funded by the European Commission under SFERA program (Solar Facilities for the European Research Area. EC Grant agreement no. 228926), Nireas-IWRC (NE YIO5030/M2/2TPATH/030809) is co-financed by the Republic of Cyprus and the European Regional Development Fund through the Research Promotion Foundation of Cyprus.

WE267 Ozonation for the reduction of the microbial load of wastewaters

J.M. Sousa, Faculty of Engineering of University of Porto / Chemical Engineering; M. Silva, Faculty of Engineering of University of Porto - FEUP / LCM Laboratory of Catalysis and Materials Associate Laboratory LSRE LCM; C.M. Manaia, CBQF Centro de Biotecnologia e Quimica Fina; A.M. Silva, Faculty of Engineering of University of Porto - FEUP / LCM Laboratory of Catalysis and Materials Associate Laboratory LSRE LCM; O.C. Nunes, Faculdade de Engenharia - Universidade do Porto / LCEIB - Laboratorio de Engenharia de Processos Ambientais Biotecnologia e Energia.

Municipal wastewater treated by conventional methods contains bacterial densities up to 10⁶ CFU/ 100 mL. To avoid environmental and health risks, the microbial load of treated wastewater should be very low, mainly if its reuse is envisaged. Ozonation is an advanced oxidation processes (AOP) currently used to disinfect wastewater by its strong biocidal action. In this study, the ozonation of wastewater to V. fischeri was carried out at fixed levels of ozone during the effectiveness of ozonation on the reduction of the microbial load of treated wastewater. A synthetic wastewater containing a mixture of microorganisms (Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Acinetobacter johnsonii, Rhodotorula rubra and spores of Bacillus cereus and Aspergillus niger) was used as a surrogate of secondary treated wastewater. The initial density of each tested bacteria and fungi was of 10⁴ CFU/100 mL and 10⁵ CFU/100 mL, respectively. Ozonation assays were carried out in a lab-scale reactor, with periods of contact of 15, 30 and 60 min. The efficiency of the treatment was assessed based on the enumeration of total heterotrophs (Plate Count Agar) and fungi (Chloramphenicol Agar), faecal coliforms and enterococci (m-Faecal coliforms and m-Enterococcus count Agar), and Aspergillus niger was determined as well. Additionally, the ability of cells to recover after ozonation was assessed. Ozonation for 15 min allowed a reduction of the total heterotrophs and fungi of 3 log and 1 log, respectively. Total coliforms and enterococci were more extensively removed, being detected at densities of about 5 CFU/100 mL. However, after 3 days at room temperature, a strong microbial recovery was observed. Total heterotrophs reached 10⁵ CFU/100 mL, which is 10 times higher than their initial density of 10⁴ CFU/100 mL. Lower recovery rates were observed for the commensal bacteria (10 CFU/100 mL and 3 CFU/100 mL for total coliforms and enterococci, respectively). After 30 and 60 min of ozonation, the cell counts were below the detection limit (1 CFU/100 mL and cells didn't reactivate after 3 days of incubation, demonstrating the effectiveness of ozonation for wastewater disinfection. Acknowledgments: Financial support for this project was funded by the Portuguese Foundation for Science and Technology (Project NORTE-07-0202-FEDER-038900 (NEPCAT), financed by FEDER through ON2 (Programa Operacional do Norte) and QREN. AMTS acknowledges the FCT Foundation of Cyprus.
WE268 Photocatalytic decomposition of 5-fluorouracil by treatment with TiO2 nanoparticles: Kinetics, Transformation Products and routes
A. Koltasikoudi, M. Sikotou, E. Evgenidou, D. Lambropoulou, Aristotle University of Thessaloniki / Chemistry
Abstract In the last years, cytostatic drugs’ demand in developed countries has grown considerably, due to the high incidence of cancer. Given the concern over the risk posed by the presence of these compounds in water bodies, and hence the possible impacts on public health and aquatic ecosystems a great deal of research is being carried out on technologies for their removal. Among them, photocatalytic treatment process using semiconductors like titanium dioxide (TiO2) has emerged as a promising nanotechnology for treating waters contaminated with highly recalcitrant organic contaminants, like cytostatic drugs. In this light, this study explored the application of heterogeneous photocatalysis (TiO2 in suspension) for the degradation of 5-fluorouracil (one of the most commonly used cytostatic drugs worldwide) in aqueous matrices. For the heterogeneous photocatalysis of 5-fluorouracil, different type of TiO2 catalysts have been tested and compared with Degussa P-25. Different factors that affect the photocatalytic process were compared: irradiation source, load of catalyst, initial concentration of 5-fluorouracil and pH. Under optimal conditions (100 mg L⁻¹ TiO₂ at pH 5.8), 10 mg L⁻¹ 5-fluorouracil can be removed within 45 min (k=0.06 min⁻¹) by using Degussa P-25. 5-fluorouracil was found to be decomposed by near-surface ‘OH free radicals produced from valence holes (h⁺). Since little knowledge exists regarding how different reactions of active ROS in photocatalytic systems have a potent effect on the removal of micropollutants, the main 5-fluorouracil phototransformation pathways observed to be hydroxylation and fragmentation. Finally, mineralization and ion analysis was performed and a feasible transformation mechanism is proposed.

WE269 Micropollutants removal by algae grown on source separated wastewater
A. Lameenhoff, Wageningen University / Environmental Technology; A. Butkovskyi, Wetsus; A. de Wilt, K. Tuatet, Wageningen University; H. Hernandez, Universidad Nacional; T. Fernandes, Netherlands Institute of Ecology (NIOO); G. Zeeman, Wageningen University
The global increase in human population and concurrent shortage of natural resources increases the interest in mining of commodities present in waste streams. For example, household wastewater is a potential source of energy, organic matter and nutrients. Source separated collection of toilet wastewater (black water) makes recovery of organic matter and nutrients economically feasible because of their concentration in a relatively small volume. As a result, separated wastewater treatment systems have gained increased interest. Treatment of black water in a compact processes, this paper also investigates in a systematic and quantitative way the effect of suspended particulate matter and selected inorganic and organic matrix constituents on both the adsorption-desorption equilibrium and initial photocatalytic degradation rate of 5-fluorouracil in water. The transformation products generated during the treatment were investigated and characterized by means of liquid chromatography coupled mass spectrometry. The main 5-fluorouracil phototransformation pathways observed to be hydroxylation and fragmentation. Finally, mineralization and ion analysis was performed and a feasible transformation mechanism is proposed.

WE270 Greywater as a sustainable water source: an integrated treatment approach coupling photocatalysis and soil columns.
T. Velegraki, Aristotle University; S. Tsourakashidou, Aristotle University of Thessaloniki; E. Diamadopoulos, Technical University of Crete; I. Poulios, Aristotle University of Thessaloniki
Given the worldwide water scarcity and future demands, the debate on the utilization of recycled wastewater as an alternative water source is gaining increasing attention. One major source of highly reclaimable water, particularly important for water-stressed nations, is the urban wastewater generated from washing activities in a household (laundry, showers, hand basins etc.) which is generally referred to as “greywater” and accounts for up to 75% of domestic wastewater. Bearing in mind that greywater generally has a lower organic load and pathogen content than municipal wastewater which includes additionally the respective streams from toilets and kitchen sinks, it may be considered as an ideal candidate for decentralized treatment and reuse systems. The present study focuses on the application of an integrated system employing titanium dioxide (TiO2) photocatalytic treatment technology and soil remediation for the detoxification of simulated greywater. Greywater was synthetically produced in the laboratory, taking into consideration the physical, chemical, and biological characteristics of actual greywater samples taken from a group of households. The sampling campaign was applied at three different sources of greywater i.e. hand basin (COD = 250±140 mg/L; pH =7±0.14), shower (COD =347±274 mg/L; pH =7±0.17) and washing machine (COD =3343±1790 mg/L; pH =6±7±0.98). A simulated effluent was produced composed of various commercial products such as laundry detergent, toothpaste, shampoo and soap. An optimization study was conducted on the photocatalytic degradation of synthetic greywater effluent in order to investigate the influence of various experimental parameters on the degradation kinetics. Results showed that ca. 47% organic content abatement was achieved after 180min of photocatalytic reaction. The pre-treated effluent was collected and introduced into soil columns so as to assess the efficiency of the land filter method in producing an effluent of high quality for subsequent irrigation purposes. Results showed that the pre-treatment followed by a physical/biological (soil columns) polishing step is a highly efficient treatment approach, as it generates an effluent of substantial quality that is safe for subsequent discharge.

WE271 Can veterinary antibiotics affect constructed wetlands performance during treatment of livestock wastewater?
C. R. Almeida, CIMAR University of Porto; A.F. Ferreira, University of Azores / Biology; F. Santos, I. Lourinha, CIMAR University of Porto; M.C. Basto, CIMAR and Faculdade de Ciências da Universidade do Porto; A.P. Mucha, CIMAR – University of Porto
Constructed wetlands (CWs) can be used to reduce the various pollutants present in livestock wastewater, such as organic matter, nutrients and metals. Very recently these systems have also been used to remove the so called emergent pollutants. These pollutants can be harmful for both microorganisms and plants, which are key players in CWs removal processes. Therefore, the influence of emergent pollutants, namely antibiotics, on the removal of pollutants from livestock wastewater should be assessed, being this the main objective of the present work. Microcosms (0.4 m x 0.3 m x 0.3 m), simulating CWs, were assembled with Phragmites australis to treat livestock wastewater doped or not with 100 μg/L of enrofloxacin or of ceftiofur, two antibiotics commonly used in livestock industry. Wastewater was treated during 8 one-week cycle, being afterwards removed and replaced by new wastewater (doped or not). At weeks 1, 2, 4 and 8 treated wastewater was collected and removal rates of nutrients (ammonia and phosphates), metals (Zn, Cu, Fe and Mn), organic matter (chemical oxygen demand (COD) and biological oxygen demand (BOD)), and solids (including total suspended solids (TSS)), as well as, of veterinary antibiotics (enrofloxacin and ceftiofur) were determined. High removal rates (up to 90% depending of the parameter) were observed independently of the presence of the veterinary antibiotics, which were also significantly removed from the wastewater. Generally, measured parameters presented values lower than those expressed in the legislation for wastewater discharge. Present results indicate that, in tested conditions, veterinary drugs presence, namely enrofloxacin and ceftiofur, did not influence significantly the biochemical processes that occur naturally in CWs during treatment of livestock wastewater. Therefore, CWs are a valuable alternative to remove pollutants, including antibiotics, from livestock wastewater, reducing the risk the release of these effluents might pose to the environment. In addition, this technology can be an efficient/economically viable technology to meet the current wastewater reuse challenges. Acknowledgments: To European Regional Development Fund through COMPETE - Operational Competitiveness Programme and national funds through FCT, under PEst-C/MAR/LA0015/2013 and ECORISK (NORTE-07-0112-FEDER-000054), co-financed by North Portugal Regional Operational Programme (ON.2 – O Novo Norte), under National Strategic Reference Framework.

WE272 Modeling Combined Technological, Environmental and Economic Considerations in Domestic Sludge Reuse via the Analytic Hierarchy Process (AHP)
I. Kalavrouziotis, HELLENIC OPEN UNIVERSITY / SCHOOL OF SCIENCE AND TECHNOLOGY; A. Bick, Bick & Associates; G. Oron, Ben-Gurion University of the Negev / Blaustein Institutes for Desert Research Zuckerberg Water Research Center
An optional method of using recycling and reuse efficiently of sludge is presented. Sludge can be originated from a number of sources, although the one obtained from wastewater is the most common one. It comprises around 30% percent of the wastewater treated by activated sludge or other similar methods. It always creates
problems related to the environmental. A multi-objective function is defined. It consists of treating the sludge by four alternatives: (i) disposal of the sludge untreated to the sea; (ii) incineration of sludge for energy generation; (iii) treatment of the sludge via anaerobic digestion, and; (iv) using the treated sludge as a soil amendment, primarily for agriculture. Comparisons of the treatment and disposal method were made. Comparison is based on the Analytic Hierarchy Process (AHP) methodology. The comparison criteria included similarly also four criteria (more can be used). These criteria included the cleanness of the sludge (emission criteria), the cost of treatment according to the final product, benefit to society in terms of energy generation and benefits to agriculture in terms of food production. There is no doubt that the solutions will be location dependent however, they demonstrate the options in each region. Key Words: Sludge; Sludge treatment; Reuse; Recycling; Benefits; Analytical Hierarchical Processes (AHP).

WE273 Removal of Carbamazepine by Phragmites australis and its endophytic bacteria
A. Sauvetre, Helmholtz Zentrum Muenchen Deutsches Forschungszentrum / Microbe Plant Interactions; P. Schroeder, Helmholtz Zentrum Muenchen / Microbe Plant Interactions
Carbamazepine is an antiepileptic and mood-stabilizing drug used widely in Europe and North America. In the environment, it is found as a persistent and recalcitrant contaminant, being one of the most prominent compounds in the list of hazardous PPCPs found in Waste Water Treatment Plants (WWTPs). Phragmites australis is one of the most abundant wetland and endophytes have been found to be used as bio-inoculants for bioremediation of difficult compounds. In this study, endophytic bacteria were isolated from Phragmites plants originating from a wetland in Escobedo, Spain. The isolates were screened for carbamazepine uptake capacity under greenhouse conditions. Phylogenetic analysis based on 16S rDNA sequences reveals that the majority of these isolates belong to three groups: Proteobacteria, Actinobacteria and Bacteroidetes. Carbamazepine uptake and plant growth promoting traits were analyzed among the isolates. Regarding to their PGP characteristics, the carbamazepine uptake and degradation, and additional characters (i.e. denitrification, catalase activity) the best strains were chosen for further inoculation studies Alone or in combination, these isolates could be used as inoculants in constructed wetlands in order to enhance the phytoremediation of carbamazepine in wastewater treatment. Keywords: Carbamazepine, Common reed, Endophytic bacteria, Phytoremediation

WE274 Uptake of perfluorobutane sulfonate and perfluorooctane sulfonates in rice (Oryza sativa)
The wastewater reuse in agriculture can pose serious environmental and health problems related to the toxic and emerging pollutants in the irrigated vegetables. Laboratory studies were carried out to assess the uptake mechanism, translocation and distribution of perfluorocarboxylic compounds (PFOSs) and perfluorobutanesulfonic acid (PFBS), which is a common substitute of PFOSs, within rice plant (Oryza sativa). Short-term uptake studies (4 from 4 to 48 h), at high contaminant concentration (1 mg/L) were performed at different pH values (4.5, 5.5 and 6.5), temperature (4° and 20°C) and light (darkness and light). In a life-cycle experiment rice plants were grown in hydroponic greenhouse from the seeds to the maturity exposed to 10 µg/L and 100 µg/L of PFOS and PFBS. At maturity the plants were harvested, and roots, stems, leaves, shoot, caryopsis and edible grains were analysed separately. Moreover growth (in term of biomass) and flowering (number of flowers) were evaluated. Experiments carried out at different temperature under darkness allowed to assess the transport mechanism of uptake when the transpiration is stopped. Data suggested that the passive diffusion (simple or facilitated) represented 63% and 74% of the total flow for PFOS and PFBS respectively. Under normal growth conditions, root concentration factor of PFOS (RCF) is 65 L/kg and the uptake constant k, is 3.98 L/kg/h which are value, while uptake constant k of PFBS is measured for root is 10 times lower than PFOS one, being RCF 0.69 L/kg and k 0.115 L/kg/h. The accumulation in rice plant and roots is equivalent for PFOS (the foliation/root concentration factor, RCF is 0.81), while PFOS preferably accumulates in roots (RCF = 0.083). Differences in uptake mechanism between PFOS and PFBS have been evidenced also in tests at different pH, from 4.5 to 7. PFBS uptake is independent of the pH value, while uptake of PFOS increases with pH decreasing. Life cycle experiments showed that plants exposed to PFOS and PFBS had a better growth than plants in control experiments and this fact can be explained by considering that perfluorinated sulfonic acids can act as bacteriostatic in the Hoagland solution used as culture medium. Nevertheless even if the biomass grows at 10 and 100 µg/L of PFOS and PFBS are comparable, at higher concentration of exposition the flower fertility was significantly reduced.

Developing end-points and effect-based methodologies for characterization of emerging and pollutant effects at relevant exposure concentrations (P)

WE275 Assessment of the Chemical Quality Improvement of Drinking Water Treatment Plants based on Human Health Risk Indexes: The case of Sant Joan Despi Waterworks by incorporating membrane processes
R. López, CETAqua Water Technology Centre; A. Rubalcaba, Fundació CM Tech Centre; J. Martin, Agbar; S. González, V. Marti, Technical University of Catalonia UPC; J.L. Cortina, CETAqua Water Technology Centre
The assessment of the chemical quality improvement of drinking water treatment plants based on health risk indexes has been developed. The methodology used for risk assessment as part of this work considers systemic and carcinogenic effects caused by oral ingestion of water based on the reference data developed by the World Health Organization (WHO) and the Risk Assessment Information System (RAIS) for chemical contaminants. Series of data covering 261 chemical parameters during 5 years (2008-2012) of raw and treated water at the low part of the Llobregat River basin (NE Spain) have been used. After the application of the methodology, resulting global indexes are located below the thresholds except for carcinogenic risk for drinking water, where the risk is slightly above the threshold during 2008 and 2009 before the upgrade of the treatment works with membrane technologies. This work performed shows that the significant change in the global indexes on the global risk for all situations: Hq systemic risk based on RAIS descends from 0.64 to 0.42 for surface water and from 0.61 to 0.31 for drinking water; R carcinogenic risk based on RAIS is negligible for surface water and varies from 4.2x10⁻⁶ to 7.4x10⁻⁶ for drinking water; W systemic risk based on WHO moves from 0.41 to 0.16 for surface water and from 0.61 to 0.31 for drinking water. A specific analysis for the risk related to trihalomethanes (THMs) showed that parameters have been calculated in order to help the decision of the stakeholders in charge of water treatment works and administrations dealing with health issues, it is important not to forget that legislation (Directive 98/83/EC) is the main reference when assessing the compliance of water quality to health standards.

WE276 Embryo toxicity and glucocorticogenic activity in JDS3 samples
Y. Shao, Department of Ecosystem Analysis ESA, B. Deutmman, Institute for Environmental Research RWTH Aachen; J. Kuckelkorn, RWTH Aachen University / Institute for Environmental Research; T. Schulze, W. Brack, Health Research Institute for Environmental Research UFZ / Effect Directed Analysis; H. Hollert, RWTH Aachen University / Institute for Environmental Research; T. Seiler, RWTH
One of the key segment that caused the aquatic ecosystem pollution in Danube and many other rivers is organic compounds and their transformation products which may pose a risk to human and environmental health. To evaluate the water quality of the Danube, we present a study on which the glucocorticogenic (GC) activity and glucocorticogenic (GR) activity of Danube water organic extracts sampled during the Joint Danube Survey 3 (JDS3) were investigated using the fish embryo test and GR CALUX® assay with zebrafish (Danio rerio) and human osteoblastic cells. In this study, all 22 JDS3 samples were collected along the Danube River from downstream, through 10 countries, to the Danube Delta. These water samples were extracted by large-volume solid-phase extraction after removing suspended particulate matter. In order to provide a comprehensive and realistic insight into the bioavailable toxic potential of the surface water, fertilized zebrafish eggs were exposed to these water organic extracts in the range of relative enrichment factor (REF) from 62.5 to 10000(1/2). The result show that embryo toxicity was detectable in all 22 JDS3 samples. 2 samples gave strong toxicity. The LC50 of JDS 32 and JDS 63 was 110.5 and 173.4 respectively. Furthermore, many different malformations and teratogenic effects appeared in this study. The most common cause of lethality due to these samples was coagulation. In a second experiment we tried to detect the glucocorticogenic activity of the samples by means of the CALUX system. The glucocorticogenic activity was detected in 4 out of the 22 sample extracts(JDS 30, JDS 35, JDS 37 and JDS39). The highest activity in JDS 35 and JDS 37 was 0.23 and 0.39 ng DEX-equival/mL. In conclusion, the identity of the compounds responsible for the embryo toxicity is currently unknown. But Glucocorticoids play a pivotal role in the regulation of glucose metabolism and inflammation which may affect biological processes and therefore may cause a threat to aquatic organisms health. Although glucocorticogenic activity was observed only in a few of samples and the concentrations measured were (low)ng/L range. Chronic exposure to these samples might therefore be expected and therefore the presence of significant levels of glucocorticoids in the environment may be reason for concern. Therefore, more attention should be paid to control the discharge of pollutants in the Danube River.

WE277 Effects of a real estrogenic effluent on reproduction in the

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Wastewater treatment plant (WWTP) effluents are a well-established point of contamination through which anthropogenic chemicals enter surface water. Whole effluent testing guidelines were developed to screen these complex mixtures for acute toxicity. However, effects-based approaches for evaluating effluents or surface waters for more subtle, sublethal effects of contaminants are lacking. Of specific concern are contaminants such as pharmaceuticals and pesticides that could affect endocrine function through action on the estrogen or androgen receptors or induce effects of sex steroid synthesis. The present study employed an effects-based approach using a historically estrogenic WWTP effluent to assess potential chronic effects of contaminants on different biological pathways. Specifically, a 21 d reproduction study using fathead minnows was conducted on-site at a WWTP using a continuous flow-through testing system that delivered final treated effluent in real-time. Breeding pairs of male and female fathead minnows were exposed to control water, or three effluent dilutions (5%, 20%, and 100%). Molecular and biochemical endpoints representing key events along established adverse outcome pathways linking estrogen receptor (ER) activation, and other relevant endocrine molecular initiating events, to reproductive impairment in fish were examined. In order to compare biological effects with chemical composition and in vitro estrogenic activity of the effluent, 7 d composite effluent samples were collected for chemical analysis and in vitro estrogenic activity assays were used to construct a chemical-gene interaction network to aid in targeted gene expression analyses. Cumulative fecundity was significantly reduced in fish exposed to 100% effluent and increased in those exposed to 20% effluent (the approximate dilution factor in receiving waters). The most potent estrogen detected in the effluent was estrone, and estrone concentration trends aligned with in vitro ER activation. Male vitellogenin mRNA transcript abundance and plasma protein concentrations increased in a dose-dependent manner with effluent concentration. Although predictable biological responses to ER agonism were observed, given the nature of chemical mixtures, additional pathways were evaluated. Results of the study provide insights into the significance of pathway-based effects with regard to predicting adverse reproductive outcomes. The contents of this presentation do not constitute official EPA policy.

WE278 Exposure of zebrafish (Danio rerio) to bisphenol AF impairs reproduction and performance of offspring generation B. Koyn, R. Delin, Department of Environmental Health / Department of Environmental Health; S. Jiang, Dept Occupational and Environmental Health; B. Kim, Department of Environmental Health; K. J. Yongin University While bisphenol AF (4,4'-hexafluorosipropylene-2,2-diphenyl, BPAF), a fluorinated derivative of bisphenol A (BPA), has been detected both in the environment and biota, limited information is available on the effects of endocrine disrupting chemicals on fish. Parental and offspring transfer to BPAF in fish of concern but such information is not yet available. In the present study, endocrine disrupting effects of BPAF on reproduction in adult zebrafish (Danio rerio) and its effects on their offspring generation following parental exposure to BPAF were investigated. Adult zebrafish pairs (D. rerio) were exposed to environmentally relevant concentrations (0.5, 5, 50 µg BPAF/L) for 21 d, and the effects on reproduction, somatic index, sex steroid hormones, and transcription of the genes belonging to the hypothalamic-pituitary-gonadal (HPG) axis were investigated. The adverse effects on performances of F1 generation were further examined with or without subsequent exposure to BPAF. The average number of eggs spawned was significantly less upon the exposure to ≥0.5 µg BPAF/L. The effective concentrations for 50% of BPAF to be in the presence of BPAF (0.5, 5, 50 µg BPAF/L) respectively. The results of hormone and gene expression showed that BPAF could alter steroidogenic pathway. Parental exposure to BPAF resulted in delayed hatching when continuing exposure to 50 µg BPAF/L. The results of sperm concentrations of F0 and F1 embryos resulted in reduced survival and increased malformation rate. The results of our study showed that the exposure to BPAF could affect reproduction function of zebrafish and development of its offspring generation. Given the extensive use of BPAF and their loadings to the environment, further investigations on potential consequences in mixture situation are recommended. Acknowledgement – This study was supported by National Research Foundation of Korea (NRF; Project no. 2013R1A1A0106184).
pharmaceuticals most frequently detected downstream the WWTP of Córdoba city. *Jenynia multidentata* is a widespread native fish proposed as bioindicator in the basin. The goals of this work were: 1) to optimize a methodology for the analysis of pharmaceuticals in small organs of fish, 2) to analyze the bioconcentration and distribution of carbamazepine (CBZ) in organs of *Jenynia multidentata* and 3) to measure changes in acetylcholinesterase (AChE) activity after exposure to CBZ, as a biomarker of neurological early effects. An extraction methodology was optimized for the extraction of at least 20 pharmaceuticals in small organs of fish (25 mg dry weight of liver, intestine, muscle, gills and 10 mg dw of brain), by ultrasonication with methanol and solid phase extraction as clean-up. Separation and detection were accomplished by UHPLC-MS/MS following an already validated method. This method was applied to quantify CBZ and 2 metabolites (100 ng/L CBZ and 2-hydroxy-CBZ) in organs of *J. multidentata*(total weight: 0.5 ± 0.2 g; standard length: 29 ± 2 mm) exposed to 100 µg/L CBZ in water for 48 h under laboratory conditions. Matrix-matched calibration using internal standards was applied (linearity in the range 0.5-1 to 50 µg/L). Limits of detection range were 0.1-30 ng/g dw and recovery between 30-150%. Matrix effects were noticeable, being ion suppression the most important one. When necessary, dilutions were made to diminish this effect. CBZ bioconcentration was observed in the 5 organs of exposed fish (max.: 701 ng/g wet weight in brain) as well as 2-hydroxy-CBZ (max.: 107 ng/g ww in liver). The active metabolite 10,11-epoxy-CBZ was quantified in gills and muscle (max.: 60 ng/g ww). An enzymatic extraction protocol was applied for the analysis of AChE activity by spectrophotometry in brain and muscle of *J. multidentata* exposed 48h to 0.5, 10 and 100 µg/L CBZ in water. A significant ACh effect was observed with a maximum of 60% reduction of activity at the highest concentration of 100 µg/L. The authors observed in muscles under the tested conditions. Results demonstrate that CBS can be uptake by fish from polluted water, metabolized and distributed in diverse tissues, affecting ACh activity.

WE282 Sub-lethal effects and protein profile alteration caused by the main cocaine metabolite (benzoylecgonine) in a freshwater biological model A. Binelli, University of Milan / Department of Biosciences; M. Parolini, University of Milan / Biology; A. Pedriali; i. marisa, Biology; M. Fedorova, R. Hoffmann, Universität Leipzig; D. Sheehan, University College Cork; S. Magni, C. Riha, University of Milan.

Illicit drugs represent not only a great social problem, but are also considered a new environmental problem because their use and, often, abuse release large amounts of parent compounds, and especially their metabolites, into freshwaters. One of the most commonly used drugs is cocaine, which is the second most prevalent drug in Europe (accounting for almost 30% of all cocaine users worldwide). Cocaine is rapidly metabolised in humans to benzoylecgonine (BE), which is eliminated in the urine and is only partially removed by wastewater treatment plants (WWTPs). Because no studies have previously been carried out to evaluate the possible risks due to cocaine and its metabolites in non-target organisms, we applied a multi-disciplinary approach to investigate the possible environmental risk related to benzoylecgonine (BE). Thus, the aim of this study was to investigate sub-lethal effects and the protein patterns alteration induced by BE on the freshwater bivalve zebra mussel (*Dreissena polymorpha*). Mussels were exposed under semi-static conditions for 14 days to two environmentally relevant BE concentrations (0.5 µg/L and 1 µg/L) and induced adverse effects were evaluated through the application of a suite of ten different biomarkers. We applied on bivalve hemocytes the single cell gel electrophoresis (SCGE) assay, the Comet assay (MN test) to investigate DNA injuries, while the neutral red retention assay (NRRA) was used to assess BE cytotoxicity. Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities, as well as the lipid peroxidation (LPO) and protein carbonyl content (PCC), were measured as oxidative stress indices in zebra mussel homogenates. Moreover, we carried out the redox antagonistic approach to evaluate the effect of oxidative stress potentially caused by BE on protein thiols and carbonyl groups in mussel gills. Finally, we analysed the protein expression profile in the gills of Zebra mussels through 2-DE and mass spectrometry analysis (RP-UPLC ESI-LTQ-Orbitrap). This study presents the main results obtained by this multidisciplinary approach in order to evaluate the environmental risk due to this new pollutant in a non-target organism.

WE283 Evaluation of fipronil toxicity to the freshwater midge Chironomus riparius: linking molecular-level endpoints to organism level effects H.R. Monteiro, University of Aveiro / Biology; J.L. Pestana, CESAM / University of Aveiro / Department of Biology; J. Rebeiro and C. Sarmento, Instituto de Tecnologias Politécnicas de Leiria / ESTM and GIRM; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; B. Devreese, Ghent University / Unit for Structural Biology Laboratory for Protein Biochemistry and Biomolecular Engineering LProBE; M.F. Lemos, Instituto Politécnico de Leiria / Dept Biology. Larval stages of nonbiting midges (Chironomidae, Diptera) can be found in almost any freshwater ecosystem. Due to its ubiquitous presence and its ecological relevance, *Chironomus riparius* is commonly used for toxicity testing and risk assessment of freshwater contaminants. Freshwater systems close to agricultural fields are subjected to frequent inputs of chemicals either by spray drift, runoff or leaching events. These inputs often contain sub-lethal and lethal concentrations of several insecticides, affecting non-target species and with possible adverse effects to ecosystem functioning. Among these insecticides, fipronil is a phenylpyrazole compound that has been replacing organophosphates and organochlorides in agricultural areas since the restrictions on their use. Fipronil acts on the central nervous system of insects by interfering with GABA-gated chloride and GluCl channels, causing an impairment of the regular inhibition of nerve impulses that ultimately result in paralysis and death. In this study, 8-10 day-old larvae (third instar) were exposed to fipronil at different concentrations to evaluate the toxicity of fipronil to midges, survival and sublethal responses such as total emergence, development time and adult body size were used as endpoints. In order to better understand and identify mechanisms of action of fipronil, molecular approaches such as enzymatic biomarkers, protein expression and fatty acid profiling were also used. Biochemical analysis indicated that fipronil significantly reduced larval LPO (LOEC of 0.08 µg L⁻¹) and affected viability of the adult midges at concentrations as low as 0.02 µg L⁻¹. The development time of exposed female midges was also significantly delayed. Adult body size, which is directly linked to the flying performance and fecundity of midges, was also affected by exposure to fipronil and we observed that emerged adults from fipronil treatments were significantly smaller than midges from control treatments. A comparison between exposed and non-exposed larvae revealed increased and decreased levels of proteins, protein activities and fatty acids. This up and down-regulation may uncover potential mechanisms of action that lead to indirect effects of fipronil, thus providing a molecular interpretation of the effects observed at the organism level.

WE284 Toxicity of two antifouling biocides, Irgoal 1051 and diuron, on two marine phytoplankton species N. Coquilhé, IRSTEIA Bordeaux; Fieram Nantes EPOC (LPTC); V. Dupraz, IFREMER; S. Stachowski-Haberkorn, IFREMER / BIOGEOCHIMIE; D. Méandre, R. Sussarel, L. Hanguarente, IFREMER / Phycolab; Irgoal 1051 and diuron are used to control biofouling and to protect Cornwall points. They target directly the photosystem II by inhibiting the electron transfer between Q{sub}B and Q{sub}A. As photosystem II structure does not vary much between different plant organisms and algae, numerous unwanted targets can be affected in case of environment contamination. The single and combined effects of these two compounds were investigated towards two marine phytoplankton e.g. *Chaetoceros calcitrans* (a single "wild" strain WC) and *Tetraselmis suecica* (two strains: "wild" (WT) and diuron-resistant mutant (MT)). The effects on growth, photosynthetic yield, reactive oxygen species presence and intracellular relative lipid content were assessed after a 6-day exposure to a range of concentrations from 0.01 µg L⁻¹ to 0.5 µg L⁻¹ for irgoal and from 0.5 to 5 µg L⁻¹ for diuron. Diuron induced a significant effect on the photosynthetic yield and irgoal with an increase of their doubling time by 168% (± 20) and 14% (± 2) respectively. DCFH-DA fluorescence related to ROS presence showed an increase by 94% (± 3) compared to the control for WT. The diuron-resistant strain MT showed no significant effect at the end of the 6-day exposure to diuron 5 µg L⁻¹. Micro-algae exposure to 0.5 µg L⁻¹ irgoal also showed significant results, as doubling time increased by 54% (± 4) for WC and by 19% (± 2) for WT after a 6-day exposure. Photosynthetic yield decreased by 25% (± 0) for WC and by 17% (± 1) for WT. The mutant strain was greatly inhibited at 0.5 µg L⁻¹ irgoal with an increase of 63% (± 2) and 60% (± 8) of doubling time and DCFH-DA fluorescence, respectively. Finally, a 6-day exposure to a mixture of diuron and irgoal was performed, but results are still being acquired. The sequencing of the pbsA gene of mutant strain is still ongoing. This study demonstrates the potential use of these two biocides commonly used in antifouling paints. It also demonstrates that resistance to diuron does not necessarily enables resistance to another PSII inhibitor. This highlights the need to monitor the fate of these two compounds in the environment, especially for irgoal, since it’s still authorized in many European countries.

WE285 Ecotoxicity of Ionic Liquids in Daphnia magna E. Sarasa, Universidad San Jorge; L.E. Eraso, Universidad San Jorge / Facultad de ciencias de la salud; E. Pereales, Health Science Department; E. Zarriaga, Universidad San Jorge; B. Giner, Facultad ciencias de la salud / Departamento de Química (COV). Ionic liquids are considered as dangerous and/or toxic for the environment. A green alternative to these compounds are ionic liquids (ILs), which have been studied as possible substitute in some scientific and industrial applications. These chemicals are organic salts melt at room temperature. Their unique properties, possibilities of combination, low volatility and flammability and insignificant vapour pressure, make them as a strong option to be considered as green solvents.[1,2] Nowadays there is a huge interest about these compounds and their physicochemical properties and possible applications have been widely investigated. However, the ecotoxicology assessment of this type of chemicals is still under going. In this study, we have evaluated the acute ecotoxicity of five ionic liquids (1-propylpyridinium tetrafluoroborate, 1-butylpyridinium tetrafluoroborate, 1-butyl-2-methylpyridinium tetrafluoroborate, 1-butyl-3-methylpyridinium tetrafluoroborate and 1-butyl-4-methylpyridinium tetrafluoroborate on daphnids (Daphnia magna), using concentration-response test, as assessed the EC₅₀. The ILs have been chosen in order to explore the structure-properties relationship. Results indicate that all the studied ILs affected strongly the mobility of *Daphnia magna*,
Concretely, the most toxic chemical was 1-butyl-4-methylpyridinium tetrafluoroborate followed by 1-butyl-2-methylpyridinium tetrafluoroborate, 1-butyl-3-methylpyridinium tetrafluoroborate, 1-butylpyridinium tetrafluoroborate and 1-propylpyridinium tetrafluoroborate, although there were not relevant differences between them. In general, we could conclude that the presence of an extra methyl substituent slightly increased the toxicity. [1] Pinto P.C.A.G.; Cota S.F.P.; Lima J.L.C.F.; Sarasa M.I.; F.S. Ecotoxicology and Environmental Safety, 2012, 80, 97-102. [2] Pham T.P.T.; Cho C.W.; Yun Y.S. Water Research, 2010, 44, 352-372.

WE286
Adverse effects of [bmim][BF4] and [omim][BF4] on mussel Mytilus galloprovincialis: an integrated approach to the use of stress indices

V. Tsaprali, University of Patras / Department of Biology; A. Belavgeni, S. Dailianis, University of Patras / Biology

The present study investigates the effects of two commonly used ILs ([1-butyl-3-methylimidazolium tetrafluoroborate] [bmim][BF4] and [1-methyl-3-oxycilimidazolium tetrafluoroborate] [omim][BF4]), in terms of their ability to induce lethal and pre-pathological alterations on marine mussels Mytilus galloprovincialis. The mortality test (96h), firstly performed, showed higher levels of mortality in [omim][BF4] water-treated (96h LC50 = 0.516 mg L⁻¹) compared to [bmim][BF4] water-treated mussels (96h LC50 = 134.536 mg L⁻¹), while [omim][BF4] acetone-treated mussels showed higher LC50 values (96h LC50 = 2.392 mg L⁻¹). Furthermore, the estimation of lysosomal membrane integrity (with the use of N-acetyl-l-cysteine, NAC), in terms of the degree of leakage of enzymatic activities, showed increased levels of lysosomal destabilization in mussels exposed to sub-lethal concentrations of ILs for 4 days. Based to the above results, a battery of stress indices in mussels’ hemolymph was estimated. According to the results, [omim][BF4] exposed mussels (both water and/or acetone treated) showed significantly increased levels of superoxide anion and lipid peroxidation (in terms of malondialdehyde equivalents), as well as increased levels of DNA damage, even at low concentrations (-1), while in the case of [bmim][BF4] exposed mussels, the obtained results were more mediated. The results of the present study clearly indicate ILs-induced lethal effects, as well as disturbances on different levels of organism function before mortality occurs. Since those ILs constitute a novel class of organic solvents, widely used in a range of applications either pure or in mixtures with water and/or conventional solvents, such as acetone, the present study reveals not only their environmental risk but their handling and management as well.

WE287
Ecotoxicity study of glycerol-derivatives in Daphnia magna

E. L.E. Eraso, Health Sciences Department; C.B. García, Universidad San Jorge / Facultad de ciencias de la salud; E. Pires, Universidad San Jorge / ISQCH; L.E. Eraso, Universidad San Jorge / Facultad de ciencias de la salud; E. Pires, Universidad de Zaragoza / ISQCH; B. Giner, Facultad ciencias de la salud

Glycerol is one of the most representative by-products in biodiesel synthesis, and it is widely used as a water-soluble solvent for the purification of glycerol as a product in biodiesel synthesis. However, the ability to induce lethal and pre-pathological effects and their toxicity can be seen by examining the physicochemical characteristics of these solvents have been developed and studied. Some of these solvents are toxic liquids, solvents from biomass, supercritical fluids, etc. However, in this case we want to focus on solvents from biomass which has been received much attention as renewable organic resources. It is important to highlight solvents from biomass such as lactate, furfural, furfuryl alcohol, terpenes, levulinates, etc. [1,2] In this case, we want to describe the lactate, furfural and levulinates. The hydrophobicity of these compounds is considered one of the most toxic disinfection agents, which led to hypoglycemia and the generation of reactive oxygen species. The LC50 at 24h exposure of Caenorhabditis elegans was 7 microM iodoacetic acid, but reproduction was severely reduced from 2.5 and behavior was altered from 1 microM iodoacetic acid. Acknowledgements: this work was supported by the grant CTM2012-31344 from the Spanish Ministry of Economy and Competitiveness and the EU FEDER program.

WE289
Ecotoxicity Study of Lactate and Furfural Compounds

L.E. Eraso, Universidad San Jorge / Facultad de ciencias de la salud; E. Sarasa, Universidad San Jorge; E. Perales, Health Science Department; E. Zuriaga, Universidad San Jorge; B. Giner, Facultad ciencias de la salud

In the last years, new fungal and bacterial strains, which need less than traditional solvents have been developed and studied. A variety of these solvents are toxic liquids, solvents from biomass, supercritical fluids, etc. However, in this case we want to focus on the conditions that may be considered as green solvents for their physicochemical characteristics. These characteristics can be used as paints, gums, dyes, oils, detergents, food additives, cosmetics, in pharmaceuticals, as lubricants, adhesives and precursor of other chemicals, etc. [1] In this study, an ecotoxicological study of lactate and furfural compounds has been carried out. New data about acute ecotoxicity of studied compounds in water have been obtained using Daphnia magna (OECD 202) bioassay. A relationship between the structures of their chemicals and their toxicity can be seen by examining the physicochemical characterization of the compound. Furthermore, the relationship of the toxicity and hydrophobicity has been investigated [2]; the log EC50 values of chemicals studied have been plotted against their log P values. [1] Nakagawa, Y.; Tomishige, K.; Production of 1.5-pentanediol from biomass via furfural and tetrahydrofurfuryl alcohol. Catalysis Today, 2012, 1, 136-143. [2] Horvath, I. T. Solvents from nature. Green Chem., 2008, 10, 1024-1028. [3] S. Pena-Tejedor, R. Murga, M. Sanz and S. Beltran, Water Research, 2005, 230, 197-203 [4] Jurado, E.; Fernandez-Serrano, M.; Nunez Oles, J.; Lechuga, M.; Jimenez, J.L.; Rios, F. Acute Toxicity of Alkylpolyglycosides to Vibrio fischeri. Daphnia magna and MicroalgaE: A Comparative Study. Bull. Environ. Contam. Toxicol. 2012, 2, 290-295.

WE290
Human exposure to endocrine disruptors by inhalation: contamination characterization of indoor air by chemical and biological analyses

S. Laborig, UMR METIS; L. Oziol, University of Paris-Sud / UMR CNRS; F. Alliot, EPHE UMR 7619; A. Desportes, EPHE / UMR METIS; E. Moreau-Guigon, M. Chevreul, EPHE

Humans are exposed to low levels of environmental compounds through several exposure pathways. The broadest range of contaminants is known or presumed endocrine-disrupting compounds (EDCs) that may interact in mixture with other contaminants under environmental exposure conditions. Inhalation represents a chronic and passive exposure route to EDCs in indoor air. In order to characterize the health hazard inherent to this multi-contamination, a two-track approach was carried out, by combination of chemical and biological analyses. Four dwelling places were compared for their indoor air contamination and with outdoor air, during two different seasons: an office, an apartment and a house to study the general population exposure, and a day nursery to consider a sensitive population. The broadest range of contaminants was extracted from the two atmospheric phases (gas and particulate) of indoor air. Liquid or gas chromatography-mass spectrometry analyses were developed for the quantification of 71 molecules, known or suspected to be EDCs, both in the two air phases. These compounds belong to several families with contrasted properties: pesticides, dielecitcs, flame-retardants, plasticizers, preservatives, synthetic musks, surfactants or compounds from combustion processes. Along with their chemical analysis, the endocrine-disrupting potential of indoor air extracts was performed using in vitro bioassays, i.e. two cellular transactivation tests: MELN and PC-DR-LUC cells respectively for estrogenic- and thyroid-disrupting effects assessment. Our data showed the indoor prevalence of
EDCs, and especially in the gas phase. The endocrine-disrupting potential and the contamination by target EDCs of the indoor air extracts were in accordance, except for seasonal variations. This suggests the presence of non-studied EDCs in air extracts or interactive effects between air contaminants. In this context, an effect-directed analysis approach will be carried out on biologically active air extracts. The objective will be to identify the target compounds responsible for the observed biological responses, while integrating interactions effects between contaminants in mixture.

WE291 Identifying New or Emerging Risks of Chemicals (NERCs) for the protection of workers, consumers and environment to support policy decision-making
Y. Bouwen de Bruyne, RIVM / Industrial Chemicals

Despite existing legislation to prevent or manage the risks of chemical substances, chemical risks continue to emerge on the short or long term. On the one hand, these risks can be the result of new substances, new applications, technological developments or process innovations. On the other hand adverse effects not recognized before might arise from the long-term use of existing substances. Therefore, a project coordinated by RIVM and financed by the Dutch Government was initiated in 2012 to develop a system to identify New or Emerging Risks of Chemicals (NERCs) at an earliest stage. The project aims to link new information on chemical stressors to effects for three protection goals - workers, consumers and the environment. A major difficulty is the causality between exposure and observed effects. Methodologies in finding and prioritizing NERCs for each protection goal will be developed. In addition, tools are suggested to provide feedback on how the selected NERCs in the nearby future. Despite similarities in the methodologies for the identification of NERCs, the complexity and route of exposure of NERCs also results in differences in approaches for the three protection goals. The two common crucial features of the developed procedures are (i) using various sources (e.g. scientific literature, news sites, websites, electronic databases, stakeholder networks) for searching information and (ii) the evaluation of information involving national networks of experts to assess the causality between the chemical exposure and the effect.

WE292 Assessment of toxic effects of ibuprofen and triclosan on the activated sludge by biomarkers approach
G. AMARIEF, K. Boltes, University of Alcalá / Chemical Engineering; P. LETÓN, Univ. Alcalá

Emerging contaminants present significant research interest due to their frequent detection in wastewater and to their reported toxicological properties. Usually, the toxicological studies carried out in water are carried out evaluating the negative effect of each compound individually on standard organisms at classical apical end-points based on the formal approach of dose-response relationships. Reviewing the literature, there are a limited number of publications on the toxicity of emerging pollutants on biological activity of microorganisms forming the activated sludge, and only individual validations have been performed. However, this response measured for a non-homogeneous community like that of the sludge is often unspecific. The aim of this study was to investigate the potential toxic effects involving oxidative stress of ibuprofen (IBU) and Triclosan (TCS), individually and in binary combination, on the microflora of activated sludge. For this purposes, were assessed biomarkers including enzymatic activities of catalase (CAT) and glutathione-s-transferase (GST). Activated sludge used comes from a laboratory scale search of the concentration of IBU and TCS range 0.5-8 mg/L and 0.006-0.1 mg/L, respectively. Batch toxicity experiments were performed using OXYGRAPH SYSTEM (Hansatech), for determining the activity of CAT based on the quantification of oxygen production rate. Fluorimetric technique was applied as well, using a FLUOROSKAN ASCENT FL (Thermo) for determining the activity of GST, using monochlorobimane-GSH as substrate. The results showed that ibuprofen induced an increase in the activity of CAT at low doses. However, further increasing in IBU concentration reduced CAT activity and returned to the level in the control. Similar to CAT, GST activity varied in a dose-dependent manner. Triclosan induced a lower increase in the activity of CAT and GST, but no significant changes were observed at all doses following its exposure. There were no regular changes of the enzymatic activities when the tested dose. The toxicants mixture had similar effects on the CAT and GST activities of IBU during individually assay, with an ease off tendency by the presence of TCS. Our findings can suggest that compared with individually substances, the co-occurrence conditions played a much more weakly role in realistic effect on the activities of antioxidant and detoxifying enzymes, as shown in the notable oxidative status imbalances.

WE293 Environmental concentrations of the cocaine metabolite benzoylecgonine induced sublethal toxicity in the development of plants but not in a zebrafish embryo-larval model
M. C. Catala Rodriguez, Universidad Rey Juan Carlos / Biology and Geology Physics and Inorganic Chemistry; J.P. García-Cambero, Institute of Health Carlos III / National Center for Environmental Health; H. Cortés, Universidad Rey Juan Carlos / Biology and Geology Physics and Inorganic Chemistry; Y. Valcárcel, Universidad Rey Juan Carlos / Department of PreventiveMedicine Public Health Immunology and MedicalMicrobiology

Several studies have found cocaine and its main active metabolite benzoylecgonine (BE) in the aquatic environment and drinking water derived from their high consumption by humans and the inability of water treatment processes to eliminate it. Only a couple of works have addressed BE eco-toxicology on animals and no data have been published of the ecotoxicity of BE on plants or microalgae. The goal of this publication is to provide information on the toxicity of relevant environmental concentrations of BE determined in plants to contribute to a better assessment of the risk of this substance for freshwater ecosystems. BE induced alterations in mitochondrial activity and DNA levels of fern spores at environmental concentrations (0.001 μg L⁻¹), which could disrupt gametophyte germination. However, these concentrations did not seem to alter the mRNAs expression, heartbeat rate or larval motility in a zebrafish embryo-larval model. These results agree with the allelopathic role described for alkaloids such as benzoylecgonine and their unspecific interference with plant germination. We conclude that the anthropogenic dispersion of alkaloid allelochemes may pose a risk for biodiversity and irrigated food production that should be further investigated.

Managing nanomaterials in the environment: lessons learnt to date, state of the art and future perspectives (P)

WE295 Determination of toxicity thresholds exerted by AgNPs on earthworm coelomocytes (eleocytes, amoebocytes) through viability and cytotoxicity bioassays combined with flow cytometric analysis
N. García, UPV/EHU / Department of Zoology and Animal Cell Biology; A. Irizar Loibide, Aarhus University (AU) / Department of Bioscience; F. Goñi de Cerio, Gaiker Technology Centre-Zamudio; J. Etxerbarria, GAIKER Tecnological Centre. IK4 Research Alliance; J. Martínez, Euskal Herriko Unibertsitatea / Zoöl Eta AnimZelulen DinamSaila; M. Soto, University of Basque Country / Zoology and Animal Cell Biology Research Centre for Experimental Marine Biology and Biotechnology PII/UPV/EHU

The number of applications regarding silver nanoparticles (AgNP) is widely increasing so their release into different environmental compartments such as soils is a major concern. In this research, there is a lack of knowledge on the hazards posed by these materials to soil organisms. Different species of earthworms have been used as sentinel and/or model organisms for soil health assessment due to their early and measurable responses (biomarkers) after exposures to a wide range of pollutants. Those responses can be measured in their immune cells, the coelomocytes. Recently, in vitro assays with primary cultures of coelomocytes based on the Neutral Red Uptake (NRU) assay have been developed as cost-effective tools for toxicity assessment of chemicals. However, coelomocytes are a heterogeneous group of cells with two main subpopulations, amoebocytes and eleocytes, which exhibit different sensitivity against pollutants. Therefore, the understanding of the behavior of both cell-types might allow the establishment of accurate toxicity thresholds exerted by AgNP, and to know whether the responsiveness of cell biomarkers can be compromised. Coelomocytes extruded from E. fetida were maintained in primary cultures and exposed to PVP-PEI coated Ag-NP (concentrations ranging 0-100 mg/L) and to PVP-PEI coating agent separately (0.0001-10 mg/L) for 24 h. The toxicity of AgNPs was assessed by cytotoxicity and viability assays (NRU and Calcein-AM Viability), and flow cytometric analyses were slightly to decipher subpopulation dynamics and their sensitivity against AgNP exposure. Internalization and subcellular distribution of AgNPs on coelomocytes was studied by TEM & X-ray microanalysis. PVP-PEI was not cytotoxic while coated AgNPs exerted an initial stress at low doses and severe toxicity at highest concentrations. In addition, a clear difference in the sensitivity of eleocytes and amoebocytes was detected, while eleocytes seemed not to be affected, amoebocytes showed a clear dose-response curve against AgNPs. Hence, AgNPs may have a targeted impact on amoebocytes as a result of the preferential accumulation that exerted subsequent subcellular damage. In vitro bioassays in combination with flow cytometric analysis succeeded in determining toxicity thresholds exerted by AgNPs in earthworm coelomocytes, being amoebocytes the most sensitive cell-type answering to AgNPs insult. Funded by Basque Government (Consol Res Groups; ITS10-13), UPV/EHU (UIF 11/37), MINECO (Nanomaterials).

WE296 in vitro cytotoxicity of silver nanowires to earthworm coelomocytes caused by surface adsorption
J. Kwak; Y. An, Konkuk University / Department of Environmental Science

By increasing concern of release of nanomaterials to terrestrial ecosystem, researches on the ecotoxicity of nanomaterials to the earthworms have been steadily reported. In this study, in vitro cytotoxicity test were carried out to assess the toxicity effects of silver nanowires to earthworm coelomocytes in the cellular level. With flow cytometric analysis of fluorescence after calcein-AM staining, intracellular toxicities were slightly decreased with increasing exposure concentrations of silver nanowires. The inhibited enzyme activities seem to have low relationship with cellular uptakes due to the negligible changes in the side scattering lights of flow cytometric analysis. However, the increase in the forward scattering lights
were observed in exposure groups. It indicated that adsorption of nanomaterials on the cell surfaces. In addition, images of silver nanowires adsorbed to the earthworm coelomocytes surfaces were observed by scanning electron microscope. These results indicate that intracellular esterase activities could be effected by silver nanowires adsorptions to earthworm coelomocytes surfaces rather than internalization in the *in vitro* exposure. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future Planning (2014R1A2A1A1050513).

**WE296**

Effects of nickel nanoparticles in the soil invertebrate Enchytraeus crypticus: a full life cycle test

F.C. Santos, Biology CESAM; S.J. Gomez, University of Aveiro / Department of Biology CESAM; J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience Terrestrial Ecology; M. Amorim, Universidade de Aveiro / Department of Biology and CESAM

The potential impact of Ni-nanoparticles (Ni-NPs) in the environment is still poorly understood, in particular in soil organisms. Concerns have been highlighted regarding the long-term effects of nanomaterials (NMs), often not covered in the currently available standard toxicity tests. Also, the effects of NMs on potentially more sensitive life stages (e.g. embryonic development) are not targeted by most standard toxicity tests. The aim of the present study is to investigate the effects of Ni-NPs (in comparison with Ni(NO3)2) on the soil invertebrate Enchytraeus crypticus on environmental Ni-NP (Enchytraeids) full life cycle (FLC) tests. This approach is a substantial improvement compared to the standard Enchytraeid Reproduction Test (ERT, ISO and OECD guidelines), as it provides more detailed information regarding the whole lifecycle and hence allow an identification of the most sensitive endpoint. In the FLC organisms are exposed from cocoons stage (1-2 days old) to 46 days, and the endpoints include: hatching (11 days); growth (15, 22 and 46 days); survival (25 days); and reproduction (46 days). Results showed that hatching was the most sensitive parameter for Ni-NPs exposure, indicating effects in the embryo development. When enchytraeids hatch and grow in the presence of Ni-NPs, their reproductive output seemed to be less affected, in comparison with the effects on the standard test where adults are exposed for 21 days. This could indicate a response mechanism in terms of number of juveniles produced as a trade-off. Last, the refined information in the FLC, compared to the traditional test, allows for a better refinement of new research questions which enables a mechanistic understanding. **Keywords:** nickel nanoparticles, soil, full life cycle, hatching

**WE297**

The toxicity of particulate (nano and micro scale) and ionic forms of copper to the marine horse mussel (Modiolus modiolus)

H.M. Alnashiri, Heriot-Watt University / School of Life Sciences; M.G. Hart, Heriot-Watt University / Centre for Marine Biodiversity and Biotechnology School of Life Sciences; T.F. Fernandes, Heriot-Watt University / School of Life Sciences Copper oxide and copper nitrate nanoparticles (CuO NPs) were used to assess the toxicity of CuO NPs within benthic filter feeders including different size fractions (nano, micro and salt). This approach is useful in many applications, such as batteries, semiconductors and ink materials. The increased usage of CuO NPs will likely result in a increased release into the environment, especially the marine environment. Furthermore, there is a lack of understanding of the toxicity of CuO NPs on environmental species and humans, particularly marine benthic filter feeders where data are particularly lacking. Therefore, the purpose of the current study was to assess toxicity of CuO NPs on the benthic filter feeders, M. modiolus, chosen for its conservation importance and for its tendency to form reefs which support other species. The mussels were exposed to different concentrations (5, 10, 15 and 20 μg/L) of different forms of particulate CuO (NPs, MPs), and copper in the form of a salt (CuSO4), along with the control (medium only), for 72 hours. The Comet assay was used to assess DNA damage in the gill cells of the mussels, superoxide dismutase (SOD activity assay) was used to assess oxidative stress. Flow cytometry was used to assess the viability of the cells and the thioarbitric acid reactive substances (TBARS assay) was used to measure the extent of lipid peroxidation in gill membranes. The results indicated that all forms of copper (particulate nano and micro) and CuSO4 had the potential to cause DNA damage in the gill cells of M. modiolus in a dose-related manner, at concentrations that can be considered environmentally realistic. Similarly, SOD activity was observed to have increased and cell viability decreased in the cells of M. modiolus exposed to all forms of copper, indicating increased oxidative stress. Finally, lipid peroxidation was increased in all exposed mussels. Overall, all forms of copper (nano, micro and salt) can potentially be toxic to M. modiolus even at low concentrations, although CuO NPs were found to be the most toxic to M. modiolus in all the experiments conducted. **Keywords:** CuO NPs, oxidative stress, DNA damage, *Modiolus modiolus*

**WE298**

Influence of condition specific aging on nanoparticle toxicity - the role of time

S. Liderveld, Universität Koblenz-Landau / Institute for Environmental Sciences; S. Seitz, Inst. for Environmental Sciences / Institute for Environmental Sciences; R.R. Rosenfeldt, University of Koblenz-Landau, Institute for Environmental Sciences / Institute for Environmental Sciences; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment Engineered nanoparticles may enter aquatic ecosystems via various pathways such as wastewater treatment plant effluence. Once in the system these particles can undergo surface modifications including, for example, the seemingly unavoidable coating by natural organic matter (NOM). This process affects the biological properties of nanoparticles both instantaneously and over time. However, the aging of nanoparticles under different environmental conditions has rarely been considered. In this context, the present study investigates the acute and chronic effects of a model nanoparticle, i.e. titanium dioxide (nTiO2, 99.9% anatase, -100 nm), on Daphnia magna, depending on environmental properties. First, 96-h acute toxicity tests at different concentrations were conducted for 6- and 21 days with varying ionic strengths (MilliQ water (0 mM/L) or ASTM-medium (9.25 mM/L)) as well as in the presence and absence of natural organic matter (±NOM). Second, 21-d chronic experiments with 0 and 3-d in ASTM-medium (±NOM) aged nTiO2 were carried out. The acute toxicity test with nTiO2 aged in MilliQ water (±NOM) did not reveal any statistically significant differences among 96-h EC50-values (0.56-1.41 mg/L). This may be attributed to a very limited nTiO2 surface modification during the aging as indicated by the similar initial particle size distributions. In contrast, the 6-d aging of nTiO2 in ASTM-medium in absence of NOM caused a distinctive particle agglomeration followed by a fourfold decrease in toxicity, compared to 0-d aging. This is also supported by the findings of the chronic experiments with 3-d aged nTiO2 in ASTM-medium +NOM, which showed reduced EC50 values (1.30-2.94 mg/L) compared to 0-d aged nTiO2 and also a transfer of these particles up through the food chain. In addition, all of these studies strongly indicate that the aging of nTiO2 modifies the toxicity of nTiO2 in a condition-specific (aqueous surrounding and time dependent) manner, while the underlying mechanisms remain unclear.

**WE299**

Trophic transfer of metal nanomaterials - should we be concerned?

S.R. Tangiraju F.R. Khan, Roskilde University / ENSPAC; H. Selek, Roskilde University / Dept Environmental Social and Spatial Change

Engineered nanomaterials (ENMs) are used in a wide range of products worldwide, such as inks, plastics, consumer products, lubricants, electronics and bioactive coatings. These particles will ultimately end up in the aquatic environment and have been shown to be taken up by a variety of species; however research on potential trophic transfer of metal ENMs is limited. In the present study, the toxicity of nTiO2 in full life cycle (FLC) tests was observed in exposure groups. It indicated that adsorption of nanomaterials on earthworm coelomocytes surfaces were observed by scanning electron microscope. The exposure groups included: 0-d with CuO NPs added to the control (medium only), for 72 hours. The Comet assay was used to assess oxidative stress. Flow cytometry was chosen for its ability to comment on the same issue in a related manner, at concentrations that can be considered similar, 1.41 mg/L). This may be attributed to a very limited nTiO2 aging. This is also supported by the findings of the chronic experiments with 3-d aged nTiO2 in ASTM-medium -NOM, which showed that there was a transfer of these particles from the lower food chain level (bacteria, algae) to higher organisms (rotifers, daphnia), even though no biomagnification occurred. Other studies have been conducted with different kind of NMs within different aquatic and terrestrial environments (e.g. Zhu et al., 2010; Judy et al., 2011; Conway et al., 2014) and all have detected a potential transfer of these particles up through the food chain. In addition, all of these studies comment on the same issue – the need for further research. By investigating the current knowledge base regarding the trophic transfer of metal ENMs, an overview of recent studies, results, concerns and knowledge gaps within this field will be obtained. A survey of the available data including experimental setup, organisms, particle types and endpoints will be discussed, and put into a greater context regarding relevant exposure scenarios and environmental reality. Such an overview is essential to better understand the risks associated with ENMs moving between trophic levels. **References:** Holbrook et al., 2008, Nature Nanotechnology, 3, 352-355; Boudin et al., 2008, ET&CC, 27 (9), 1958-1963; Zhu et al., 2010; Conway et al., 2014 and Judy et al., 2011; ES&T, 45 (2), 776-781; Conway et al., 2014, ES&T, 48, 1517-1524

**WE300**

Modification of nanoparticles to reduce environmental toxicity

B. Kruse, University Brodowin School of Life Sciences; T.F. Fernandes, Heriot-Watt University / School of Life Sciences

The FP7 project NOMICEX focuses on safety by design to generate nanoparticles useful for the ink and pigment industry containing modifiers to mitigate their risk to humans and the environment. Results from initial acute aquatic OECD-304 test and attention showed that the most tested metal ENMs with a panel of seven nanoparticles, zinc oxide (ZnO) and hydrophobic quantum dots (CdSe) were the most promising candidates for successful modification, showing both high toxicity and potential for modification with organic ligands. Acute toxicity tests according to OECD protocols were performed using Daphnia magna, Lamprocladus variegatus and Pseudokirchneriella subcapitata as test models, and particle concentrations of up to 100 mg/L were used; for certain particles with low toxicity an EC50 could not be established in some organisms. Modification of ZnO with a protein coating reduced the EC50 in *D. magna* and *P. subcapitata* by a factor of 171 in *P. subcapitata* and more than 300 in *D. magna*, while ZnO had not shown toxicity in *L. variegatus* prior to modification. For hydrophobic CdSe, modification
of the particle core with a ZnS shell and a silica-linked sugar reduced the EC50 in *P. subcapitata* by a factor of 3.15, in *D. magna* by a factor of 125, and in *L. variegatus* by a factor >3. *L. variegatus* locomotive behaviour was also less affected by the new modified CdSe than by hydrophobic CdSe NPs. The biokinetics of unmodified and modified ZnO NPs to *L. variegatus* and sub-lethal effects on oxidative stress in *L. variegatus* and *D. magna* are currently being investigated by assessment of cardiac and supragnadular activities in the organsisms. In parallel studies, we also found that the modified nanoparticles had reduced cytotoxicity in mammalian hepatic, phagocytic and alveolar cells. These findings suggest that the modifications established in this project may be a step towards safety by design, generating particles which can be produced at a moderate to large scale and employed by industry, with less risk to workers, consumers and the environment.

WE301

Environmental, health and safety impacts of the use of nanomaterials in the textile finishing industry, ECOTEXNANO project.

R. Villafla, Leitat Technological Center / Sustainability Unit; N. Fuentes, Leitat Technological Center; M. Escamilla, LEITAT / Sustainability Unit; J. Mota, Leita Technological Center / Advanced Materials Division; M. Santamaria, ITENE / Chemical Safety Division

In response to competitive challenges, the textile industry in Europe should improve their competitiveness by ceasing mass production and simple fashion products and concentrating instead on higher value-added sustainable products. The use of nanomaterials is gradually increasing daily due to the new properties addressed in the EU and modified to allow for sustainable products. This new trend results in a key environmental problem due to the lack of knowledge of their health and environmental impacts and subsequent effects on the ecosystem. Within this scope, the overall aim of ECOTEXNANO project is to improve the environmental performance of best innovative solutions that are emerging with regard to technical textiles that incorporates nanoparticles in the textile finishing industry. The project addresses four textile functionalities: soil release, UV protection, antimicrobial and flame retardant; the most representative nanomaterials that are being used in textile finishing processes by functionality have been selected. Environmental, health and safety impacts are being assessed by carrying out a comprehensive Life Cycle Assessment of the manufacturing operations and Risk Assessment of the selected nanomaterials. The analysis will allow quantifying the environmental impacts of the use of nanomaterials in substitution of bulk substances, and to guarantee that these nanomaterials do not pose risk on workers and environment. Demonstration is being developed into two pilot scale trials (Spain and Italy) in order to provide evidence of best practice in the application of nano-based techniques comparing with the conventional finishing chemicals. The project results will provide the textile finishing industry user-friendly tools to support the chemical safety assessment of nanomaterials along their life cycle and to bridge knowledge gaps on nanomaterials properties, hazard and exposure. The tool will compare nanotextiles and conventional textile finishing products and quantify the achieved environmental and risks improvement. It will be the basis for further development of a network platform to share data with relevant stakeholders. In addition, an online platform will be available to be used by universities, research centers, and industry companies to quantify the environmental improvement and risk reduction achieved. Based on the results, human health and environmental EU policy such as REACH and CLP Regulation, biocidal products, BREF for textile sector will be analyzed for potential updating.

WE302

The Effects of Nanoparticles on The Freshwater Microalgae Pseudokirchneriella subcapitata

F.M. Alqahtani, Heriot-Watt University / School of Life Science; J. Kalman, School of Life Sciences; H. Johnston, Heriot Watt University / Life Sciences; V. Stone, Heriot Watt University; T.F. Fernandes, Heriot-Watt University / School of Life Science

This poster reports the results of a study assessing the effects of different nanoparticle samples on the microalgae *Pseudokirchneriella subcapitata*. The increased use of nanoparticles in a wide range of products has led to their potential release into the environment. In this study the microalgae *Pseudokirchneriella subcapitata* was exposed to different concentrations of a range of nanoparticle widely used in consumer applications. Algal growth was measured at different time points (0, 24h, 48h, and 72h) using different methods, namely optical density (OD) and fluorescence of the culture, as well as chlorophyll a, after extraction (OECD guideline 201). Results indicate a dose response effect, with increased inhibition observed with increased nanoparticle concentration. A comparison of the different nanoparticle studies was made in regards to their toxicity to the microalgae discussed in the context of their use in realistic scenarios.

WE303

QSAR models for the prediction of the adsorption of organic molecules on multi walled carbon nanotubes

E. Papa, Department of Theoretical and Applied Sciences

The investigation and prediction of toxicological and physico-chemical properties of nanoparticles (NPs), as well as of the interaction of organics with NPs surface, is a challenging topic. In silico approaches based on quantitative structure-property relationships (QSPR) can be applied to investigate ecotoxicologically relevant properties of NPs. This poster shows some examples of QSPR models generated to predict the potential adsorption of small organic molecules on the surface Multi Walled Carbon Nanotubes (MWCNTs). The organic chemicals interacting with the surface of the MWCNTs were structurally described by conventional molecular descriptors, which have been calculated by using freely available online software (PaDEL Descriptors). The QSPR models were developed in the software QSARINS by Multiple Linear Regression (MLR - Ordinary Least Squares(OLS)) supervised and external validation was performed by splitting the available experimental data into training and prediction sets. The All Subset and the Genetic Algorithm Variable Subset Selection procedures were applied to select the modelling structural information from the hundreds of molecular descriptors available in this study. Literature models generated by using Abraham descriptors were compared with the new QSPRs based on conventional descriptors. In parallel, an in depth analysis was performed. The toxicity domain of the models has shown that the current experimental information used to generate QSPR models, was not sufficient to generate reliable predictions for the potential adsorption of small biomolecules (i.e. hormones) on the surface of MWCNTs. Finally, a robust and externally predictive QSPR regression model was proposed to predict the adsorption of small organics on MWCNTs. This model is a valid alternative to the more complex literature models developed for the same endpoint using Abraham descriptors.

WE304

Quantitative (Nano)-Structure Activity models for heterogeneous metal oxide nanoparticles

E. Papa, Department of Theoretical and Applied Sciences; J. Ponti, European Commission, Joint Research Centre, Institute for Health and Consumer Protection; J. Doucet, A. Panaye, R. Brayner, Paris Diderot University / ITODYS

The current use of nanoparticles (NPs) for industrial and pharmaceutical applications has become a reason of increasing concern due to their possible toxicological activity in humans and other organisms. The lack of experimental data available on available to describe structural properties of these new materials and the lack of in vivo or in vitro measured activities for large sets of NPs, are among the main problems associated to the development of in silico models based on Quantitative (Nano)-Structure Activity Relationships (Q(N)SARs). In this poster we want to show some examples of Q(N)SARs developed to model the biological activity of heterogeneous metal oxide NPs, also highlighting the strengths and weaknesses of the performed approaches. In different datasets of heterogeneous metal oxides NPs structurally characterized by experimentally measured properties, such as surface charge, size, aggregation properties etc. were modelled to predict different responses of biological activity (i.e. cytotoxicity and cell membrane damage). The Q SAR models were developed by using several statistical methods to the development and the validation of QSARs, thus paying particular attention to the evaluation of applicability domains, to the validation of the models, and to the interpretation of the molecular descriptors. The proposed models are promising, however small applicability domain represents one of the main limits associated to these models. This is a direct consequence of the limited dimension of the available experimental data sets. Observations from this study provide additional information on which issues represent limitations and possibilities for the future improvement and application of Q SAR-based approaches to NPs. This information is useful to improve application of in silico methods to hazard and risk assessment procedures of NPs, and to perform in silico screening of NPs.

WE305

In silico approaches for the prediction of the biological activity of surface-modified nanoparticles

E. Papa, Department of Theoretical and Applied Sciences; J. Doucet, A. Panaye, Paris Diderot University / ITODYS

Different in silico approaches based on quantitative structure activity relationships (QSAR) are currently applied to investigate the potential biological activity of nanoparticles (NPs). In this poster we want to show some examples of models which can be generated to study the effect of changes in the organic coating on cellular interaction phenomena of surface-modified NPs. Different literature datasets were used to model in vitro “uptake in” and “association to” cells, of surface-linked iron oxide, and taking into account the OECD principles for surface modifications. The organic chemicals used to modify the surface of the functionalised NPs were structurally described by conventional molecular descriptors, which were calculated by using freely available online software (PaDEL Descriptors). Structural information on experimentally derived structural descriptors (i.e. NPs size, charge, and aggregation state) was as well as on the serum protein bound fraction was made available for one of the data sets. We applied multivariate analysis to investigate macro-properties of the structural datasets, and this information was useful to perform the subsequent QSAR modelling on the endpoints of interests. Different statistical approaches were used to generate QSAR models. The selection of the best combinations of descriptors was performed by applying the All Subset and of the Genetic Algorithm Variable Subset Selection procedure. The best models were validated for internal robustness and predictivity, and the external validation was performed by splitting the available experimental data into training and prediction sets. A mechanistic interpretation of the modelling descriptors was proposed in order to clarify which structural features have the highest influence on the modelled endpoints, and the...
applicability domain of the models was quantified and explored to evaluate the reliability of predictions. Finally, the poster will include some preliminary results on the prediction of cellular interactions by using the serum protein corona “fingerprint”.

**WE306 Oxidative stress of Silver nanomaterials to soft- and hard-bodied soil living invertebrates**

J.A. Mendes, Department of Biology CESAM; M.J. Ribeiro, Biology; V.L. Maria, Biology Department of Aveiro University / Biology CESAM; J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience Terrestrial Ecology; M. Amorim, Universidade de Aveiro / Department of Biology and CESAM

It is known that silver nanomaterials cause toxicity to organisms, possibly partly through oxidative stress. In the present we studied the link between oxidative stress and the reproductive effects. We compared the oxidative response between a soft and a hard bodied organism, Folsomia candida and Enchytraeus crypticus, both standard species, employing exposure to both nano silver and soluble silver salts. To get a comparable mechanism of response scenario the comparison was based on exposure at similar effect concentrations [e.g. EC20,50,80] for the reproductive output and along time (0-10 days). The measured markers were catalase, glutathione reductase and glutathione-s-transferase activities, as well as total glutathione, metallothionein and lipid peroxidation levels. The response mechanisms seem, in general, to be similar in both organisms to each of the silver forms, although more sensitive level differences between the two species. Similar activation pattern of glutathione reductase for either silver form was determined, while metallothionein increased after exposure to AgNP for both species. Differences in responses between species were observed in terms of time of events: for E. crypticus, LPO levels and enzyme changes occurred earlier (3 days) than for F. candida for AgNO3 whereas the opposite occurred for AgNP (7 days for E. crypticus and 2 days for F. candida).

**WE307 Tracing effects of NMs along their life cycle - toxicity in soil (Enchytraeus crypticus)**

F.C. Ricche, Universidade de Aveiro / CESAM Department of Biology; M.J. Ribeiro, Biology; J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience Terrestrial Ecology; M. Amorim, Universidade de Aveiro / Department of Biology and CESAM

NMotors (NMs) are used in a vast range of applications and their sustainability requires assessment. In FP7-SUN-SUSTAINABLE Nanotechnologies (contract No. 604305) one of the aims is to assess the effects of NMs that are commonly used in products and applications within all of the life cycle. Hence, in the present we assessed the toxicity of a range of NMs at different life cycle stages (pristine, in use, weathered). In this case we focused on the terrestrial compartment and the species Enchytraeus crypticus was used. The tested NMs were copper oxide (CuO), iron oxide (FeO), the organic pigment CoAlO, and the metal Cobalt Tungsten (WCo). Ecotoxicity tests followed ISO and OECD guidelines for enchytraeid survival and reproduction standardized test (ERT), improved to start with synchronised age organisms. Results showed that CuO NMs reduced survival and reproduction in dose related manner being among the most toxic. Comparisons are made between pristine and in use materials for toxicity. Further tests are ongoing including all NM life cycle stages. Acknowledgements: SUN, FP7-NMP-2013-LARGE-7 (No. 604305)

**WE308 Bioaccumulation, subcellular partitioning and metabolism of CdTe quantum dots in the marine mussel Mytilus galloprovincialis**

T.L. Rocha, University of Algarve / CIMA; T. Gomes, Norwegian Institute for Water Research (NIVA); E.G. Durigon, M.J. Bebianno, University of Algarve / CIMA

The increased production and use of Quantum Dot (QDs) in many consumer products and application in nanomedicine raise concerns about their environmental release and impact in aquatic organisms. In this context, metallothioneins (MTs) are metal inducible proteins which participate in the detoxification, storage and metabolism of metals and defense against oxidative stress. These proteins are recognized biomarkers of metal exposure in monitoring environmental pollution, but its functions in invertebrates exposed to engineered nanoparticles (ENPs) remains less known. So, the aim of this study was to investigate the high-accumulation and subcellular partitioning of CdTe and their association with MT induction and oxidative damage in the marine mussel Mytilus galloprovincialis exposed to CdTe QDs and its soluble counterpart. Mussels were exposed to 10 μgCdL⁻¹ of CdTe QDs and cadmium nitrate for 21 days (accumulation period) and transferred to clean seawater for 50 days (depletion period). Total Cd accumulation and subcellular partitioning (insoluble fraction - IF, high-molecular-weight protein fraction - HMW, low-molecular-weight protein fraction – LMW) of accumulated Cd were determined in mussels’ gills and digestive gland. Oxidative damage was evaluated in terms of lipid peroxidation by determining malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) concentrations and MT levels were measured by differential pulse polarography. Both CdTe QDs and soluble Cd accumulated with time in mussels’ tissues in a Cd form and tissue dependent manner. Similarly, the percentage of Cd distributed among subcellular fractions was also dependent on the tissue and Cd form. Mussels exposed to QDs and soluble Cd showed higher LPO levels and MT concentrations in their tissues. Cd found in LMW fraction was directly related to MT levels in mussels exposed to both Cd forms, reinforcing the importance of this metalloprotein in Cd metabolism. During the depletion period, mussels showed a similar Cd concentration and MT levels when compared to those at the end of the accumulation period. This indicates that the subcellular partitioning of QDs and their soluble counterpart provides an improvement to predict the metabolism and mode of action of ENPs in marine bivalves and the important role of MTs in the detoxification and elimination of Cd-based QDs and against oxidative stress induced by these ENPs.

**WE309 Sub-lethal toxicity of modified and unmodified nanoparticles in freshwater organisms**

J. Roy, Heriot-Watt University / School of Life Science; B.K. Gaiser, School of Life Sciences; T. Fernandes, Heriot-Watt University / School of Life Sciences

Manufactured nanoparticles are increasingly being used to improve the quality of products and materials. Health and safety of workers is an issue of great concern. Focusing on this issue, the NANOMICEX project (FP7) aims to mitigate risk and control exposure in nanotechnology, particularly for ink and pigment based industries. The research is focused on design and risk assessment of engineered nanomaterials using cost effective and environmentally friendly method, and increasing the level of safety for workers while retaining properties necessary for their applications. At the start of the project, ecotoxicology tests were performed using the model species Daphnia magna, Lumbriculus variegatus and Pseudokirchneriella subcapitata. Standard OECD protocols were followed for acute toxicity tests. Based on the market information of inks and pigments, ZnO, TiO2, CuO, CoAlO, CdSe and CdS nanoparticles were tested for toxicity. The highest toxicity was found for Ag, ZnO and hydrophobic CdSe QDs. After successful surface modification of ZnO with a protein coating and CdSe quantum dots with a shell containing ZnS and sugars linked by silica, the modified particles were found to be less toxic than their unmodified counterparts in all test organisms. In this study, further experiments were undertaken to find out whether the reduced toxicity observed also applied to sub-lethal effects such as locomotive behaviour of L. variegatus and markers of oxidative stress in L. variegatus and D. magna tissue, since nanoparticles containing metals are known to cause toxicity by impacting oxidative defences. Organisms were exposed to unmodified and modified ZnO and CdSe QDs, as well as metal ions. While 10 mgL⁻¹ of CdSe QDs caused a change in L. variegatus locomotive behaviour, unmodified ZnO and CdSe QDs were tested for toxicity. Toxicity in zebrafish L. variegatus were also unaffected by either type of ZnO. Exposures and sample collection for catalase (CAT) and superoxide dismutase (SOD) activity after 4 h (D. magna) and 2, 4 and 24 h (L. variegatus) were also studied. At this point, the modified nanoparticles appear to be safer to use than their unmodified counterparts, and nanoparticle modification with biomolecules could be an important step in making nanotechnology safer and more environmentally friendly. Further research will provide insight into the mechanism of particle toxicity and its prevention, and can be used to establish structure-activity relationships.

**WE310 Testing the effect of nanomaterials in worms - lessons learned from in vitro and in vivo studies**

J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience Terrestrial Ecology; P. Irizar, Aarhus University AU / Biosciences; J. martiaydas, Aarhus University, DMU / bioscience; M. Amorim, Universidade de Aveiro / Department of Biology and CESAM

The impact of toxicity of nanomaterials (NMs) on living species is still poorly understood, in particular in soil organisms. This lack of understanding is partly due to technical difficulties in the actual testing, partly due to a lack of understanding the fundamentals of how NMs interact with biological systems. The aim of the present poster is to provide an overview of the experience gained when testing the possible effect of NMs on the model earthworm species Eisenia fetida. In this work, focus is on the difference in activity of different ZnO-Si-Glucose tested toxicity of NMs compared to testing traditional chemicals. This includes exposure considerations regarding the best spiking approach, exposure assessment requirements, and the optimal experimental design. In relation to the assessment of the biological responses caused by NMs, the focus is how in vitro studies can support commonly performed in vivo studies when dealing with NMs. In vivo studies can for example support the identification of mechanisms of actions and toxicity pathways which provide an insight into the interactions between NMs and organisms. Practical guidance (e.g. most appropriate methods of adding NMs to the media) and a proposed way forward are suggested, referring to the best practices and homogenisation with OECD and other standardisation purposes.

**WE311 Data requirements for creating quantitative relationships between engineered nanomaterial related characteristics and exposure or effect endpoints**

J.T. Quik, RIVM / DMG; D. De Zwart, RIVM / Centre for Sustainability Environment and Health; M. Bakker, RIVM; I. Gosens, RIVM / DMG; H. Marvin,
Currently there is data available on different aspects of the risk assessment of engineered nanomaterials (ENMs). However, in the future all not engineered nanomaterials can undergo experimental testing to acquire the data needed for risk assessment. For this reason smart methods for read across from previous measurements or use of quantitative models is needed. At this point there are many predictive exposure assessment models. Several effect studies have resulted in the first specific exposure data distributions. In our current study we have reviewed the current literature to indicate the known qualitative and where possible quantitative relationships between engineered nanomaterials and endpoints that are related to exposure assessment or effect assessment. This resulted in a set of characteristics describing ENMs, the suspension matrix and the interaction between the ENM and the matrix. These ENM-specific research is refined which will report data on each of these categories. Ultimately there is a need to have more data available that describe the experimental conditions, this can be done using some of the currently available data systems and tools for reporting experimental data on ENMs.

WE312 Toxicity of selected nano-objects and their aggregates and agglomerates (NOAA) to freshwater invertebrates
V. Ricottone, Heriot-Watt University / School of Life Science; T.B. Henry, Heriot-Watt University / School of Life Sciences; V. Stone, Heriot Watt University; T.F. Fernandes, Heriot-Watt University / School of Life Sciences
This research funded by the European FP7 project SUN “Sustainable Nanotechnologies” which aims to address the entire lifecycle of nano-objects and their aggregates and agglomerates (NOAA) (from cradle to grave) creating an integrated approach for the long-term sustainability of nanotechnologies through the development of safe processes for production, use and end-of-life processing of nanomaterials and products, as well as methods reducing both adverse effects and exposure to acceptable levels. Aquatic exposure studies were conducted on two model species: the cladoceran Daphnia magna and the snail Lymnaea stagnalis. The experiments have started assessing the ecotoxicity on the aquatic ecosystem of prioritized pristine engineered NOAA (Org. Pig.-Red254, WC-Co, MWCNTs and CuO) among the 8 different SUN nanomaterials. The initial studies were focused on the controlled release of toxicity from either NP s or juveniles (7-9 day old) of the pond snail L. stagnalis exposed for 96h at 20°C to Cu, either in the form of CuO NPs or as aequous salt, CuSO\textsubscript{4}\text{\(\cdot\)}\textsubscript{5}H\textsubscript{2}O. L. C50 values estimated were, respectively, 2500 μg L\textsuperscript{-1}, Cu and 5.5 μg L\textsuperscript{-1}, Cu, indicating a greater toxicity on L. stagnalis of CuSO\textsubscript{4} than CuO NPs. Screening tests were conducted for the remaining prioritized nanomaterials using the crustacean D. magna, in order to assess the threat these NOAA might pose onto the ecosystem before proceeding with further investigations. Overall results indicate a moderate toxicity for Org. Pig.-Red254 and a much lower toxicity for the other two NOAA, WC-Co NPs, and MWCNTs. Respectively, L(E)C50 immobilization values on D. magna for Org. Pig.-Red254, WC-Co NPs are: 1900 μg L\textsuperscript{-1}, Org. Pig.-Red254, 3370 μg L\textsuperscript{-1}, WC-Co NPs. For MWCNTs, in the range of exposure concentrations was only possible to detect UV-vis spectroscopy, dynamic and phase analysis light scattering (DLS and PALS respectively). We determined the relative accumulation of these two forms of silver NMS in this in vitro model using inductively coupled mass spectrometry (ICP-MS) and electron microscopy studies. Subsequent to bioaccumulation studies, we evaluated the biotransformation activity (EROD) and oxidative stress in terms of glutathione and lipid peroxidation (TBARS). Characterisation studies suggested a change in surface chemistry and solvent solubility of the materials in the presence of serum, which was common for both the NMs used. This was confirmed by UV-vis spectroscopy which showed the characteristic surface plasma resonance (SPR) absorbance peak at ~410 nm in both media. Overall, our studies indicate the 3D spheroid models as an efficient and robust model to study bioaccumulation and potential toxic effects of different forms of engineered NMs. Keywords: Silver, nanomaterials, 3-D liver spheroids.

Rational design of chemicals and pharmaceuticals and other input prevention measures towards sustainable technological development (P)

WE315 Designing nanomaterials towards a sustainable nanotechnology: and example with polyelectrolyte-surfactant nanomaterials
J. Lopes, University of Aveiro / CESAM Biology Department; G. Chagas, Federal University of Bahia; S. dos Santos, EMPA Technology Society Lab; C. Duarte, F. Antunes, University of Coimbra; E.M. da Silva, Instituto de Biologia da UFBa / Institute of Biology, Department of Botany; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM
Complexes constituted by polyelectrolytes and oppositely charged surfactants are a class of materials with growing interest due to their importance and applications for gene therapy, drug delivery, care products, among others. As their biological environment can modulate its biological identity and physical chemical properties. The objective of this work are: i) to characterize the evolution of PC in the transition through different biological media, in terms of both changes in composition and physicochemical properties, ii) develop methodologies that can define the specific enrichment of the PC surface, and iii) integrate MS-based PC data to develop nanointeraction tools for nanosafety. The results presented here will contribute to define a systematic characterization of PC, fundamental to understand nano-cellular interactions and establish adequate in vitro and in vivo models for NP risk assessment. Many studies have addressed the properties and composition of the corona in biological fluids or plasma, but not during the transition from one biological environment to another, tackling the dynamism in the corona across different environments. This work seeks to define the potential characteristics and fluid behavior of the PC evolves across distinct biological media by knowing its physicochemical properties and identity. This knowledge should help to evaluate and even predict cellular responses to NPs. We proposed that by evaluating the proteins in PC, how protein adsorption changes across different biological media, and how this affects NP physicochemical properties, this knowledge may serve to address the NP-cellular interaction in biological environments. The results offer new perspectives and improve methodologies to study nano-bio interactions with more complex environments and to develop nanointeraction tools with prediction capability.

WE314 Uptake, distribution and toxicological effects of two different silver nanomaterials in 3-D in vitro fish cultures
A.N. Jha, Plymouth University / Biological Sciences; T. Vicari, Plymouth University / School of Biological Sciences; M. Baron, School of Biological Sciences; R. Maunier, Plymouth University / Biological Sciences; A. Latorre, University of Nottingham / Nottingham Nanotechnology Nanoscience Centre; G. Range in Biobest requirements and in vivo toxicological models. The results offer new perspectives and improve methodologies to study nano-bio interactions with more complex environments and to develop nanointeraction tools with prediction capability.

WE313 Evolution of nanoparticle-protein corona, learning lessons from quantitative proteomics for nanosafety
S. Cristóbal, Linköping University / Department of Chemical and Biological Engineering
Nanosafety is today focused on investigating the key elements that could provide the essential and sufficient information to provide safety for people and the environment. One of those key elements is the understanding of the nano-bio-eco interface. The state-of-art of mass spectrometry could build a bridge to ravel the key features of nanoimpact on the environment, biological fluids or cell content and promote a framework to evaluate the impact of any future nanotechnological innovations. The nanosafety of a nanoparticle (NP) should be studied through its evolution rather than at the stage of newly synthesized entity. The nature of the particle corona (PC) in contact with different biological environments has been extensively reported. Nevertheless, less few studies addressed the evolution of the PC in terms of how the transition from an extra- to intra-cellular environment can modulate its biological identity and physical chemical properties. The objective of this work are: i) to characterize the evolution of PC in the transition through different biological media, in terms of both changes in composition and physicochemical properties, ii) develop methodologies that can define the specific enrichment of the PC surface, and iii) integrate MS-based PC data to develop
were obtained by changing the number of repeating units (m) of NaPA (NaPA_m) which was always at a concentration of 10 mM of repeating units, the counterion of the surfactant (bromide-DOTAB or chloroide-DOTAC) and the concentration of DOTAC (3, 5, and 7 mM): NaPA_m/10DoTAC3; NaPA_m/10DoTAC5; NaPA_m/10DoTAC7; NaPA_m/10DoTAC9; NaPA_m/10DoTAC11; NaPA_m/10DoTAC13; NaPA_m/10DoTAC15; NaPA_m/10DoTAC17; NaPA_m/10DoTAC19; and NaPA_m/10DoTAC21. The lethal and sublethal toxicity of these NMs was assessed by exposing the bacteria Vibrio fischeri, the microalgae Pseudokirchneriella subcapitata, and the cladoceran Daphnia magna to serial concentrations of each NM. The following endpoints were evaluated: bioluminescence inhibition, growth rate and cumulative mortality, respectively. As well, the physical parameters of hydrodynamic size, index of polydispersity, and zeta potential were evaluated for the highest concentration of each NM. In general, the obtained results showed that the NMs composed of DOTAC at a concentration of 3 mM exerted the lowest toxicity to the three tested species, while those composed of NaPA with 2000 repeating units (NaPA2000) were among the most toxic. Therefore, aiming at a greener and sustainable development of nanotechnology, it is suggested that, for this type of NM, complexes formed by DOTAC (with the chloride counterion) at concentrations of 3 mM should be target to be developed.

WE316 Classification of baseline toxicants in fish to replace in vivo testing with reliable QSAR predictions
M. Nendza, Analyticals Laboratory; M. Müller, A. Wenzel, Fraunhofer-Institute for Molecular Biology and Applied Ecology
Reliable QSAR predictions are specific by mode of action (MOA) and compounds with different MOA must be predicted with different QSARs. Functional similarity of chemicals combines toxicological knowledge (which toxicity pathways are stimulated in which species under which exposure conditions) with chemical expertise (which chemical structures and physico-chemical properties are involved in which interactions) to discriminate between baseline and excess toxicants. The objective is to identify as many baseline toxicants as possible as their acute fish toxicity can be predicted with sufficient accuracy from their log Ka and, hence, their in vivo testing may be waived. In contrast, excess toxicants, and chemicals that cannot be reliably classified as baseline toxicants, require further in silico, in vitro or in vivo assessments. Established tools, such as structural alerts, are used to indicate MOAs that are typical causes of excess toxicity. Verhaar classifications are supplemented with additional chemical attributes and physico-chemical property thresholds to cover a larger range of compounds within the baseline toxicity domain. A stepwise approach was developed to first detect potential excess toxicants and then to posteriorly identify true baseline toxicants. Our objective was to identify baseline toxicants (true negatives) in a precautionary way, not ignoring possible excess toxicants (true positives). At the same time, we tolerate a certain fraction of false positives, i.e. baseline toxicants without specific effects that may be tested instead of predicted. Classification statistics show an overall accuracy of 75.5%. The sensitivity is 96.3% and confirms the precautionary approach of excess toxicants, i.e. only four mild excess toxicants falsely predicted to have baseline effects. The specificity is 70.5% and indicates potential for improvement. The classification of 56.9% of the compounds of the EPA Fathead Minnow Acute Toxicity Database (EPAFHMF) as baseline toxicants suggests that, if this is representative of industrial chemicals, more than 50% of acute fish toxicity testing could be replaced with reliable QSAR predictions.

WE317 Oxidative stress toxicity a gateway for the design of safer chemicals using two model fish species: zebrafish and fathead minnow.
J. Corrales, Baylor University / Environmental Science; L.A. Kristofco, Department of Environmental Science Center for Reservoir and Aquatic Systems Research; J. Yates, Oregon DEQ / Laboratory; W.B. Steele, Baylor University; M.S. Williams, CSIRO / Department of Chemistry and Geochemistry; M. Mills, University of Washington; E.P. Gallagher, Department of Environmental and Occupational Health Sciences; T. Kavanagh, N. Simcox, University of Washington; L. Shen, Yale University / Center for Green Chemistry and Green Engineering; E. Beach, J. Zimmerman, Yale University; J. Corrales, Baylor University / Environmental Science; M. Mullins, Baylor University; J. Yates, Oregon DEQ / Laboratory; E.P. Gallagher, Department of Environmental and Occupational Health Sciences; E. Thompson, J. Kostal, George Washington University; S. Williams, Environmental Studies; J. Kostal, George Washington University / Chemistry; J. Zimmerman, Yale University; P. Anastos, Yale University / Center for Green Chemistry and Green Engineering; B.W. Brooks, Baylor University / Dept of Environmental Science
Despite lack of experimental health and safety data in over 85% of the approved commercial chemicals, toxicologists, negative, i.e. only four mild excess toxicants falsely predicted to have baseline effects. The specificity is 70.5% and indicates potential for improvement. The classification of 56.9% of the compounds of the EPA Fathead Minnow Acute Toxicity Database (EPAFHMF) as baseline toxicants suggests that, if this is representative of industrial chemicals, more than 50% of acute fish toxicity testing could be replaced with reliable QSAR predictions.

WE319 Understanding Oxidative Stress Response (ROS) and oxidative stress (OS) are associated with adverse effects such as inflammation suppression, cancer, aging, rheumatoid diseases, cardiovascular disease and Parkinson’s and Alzheimer’s diseases. OS is associated with an array of structurally diverse chemicals and may proceed by a variety of toxicity pathways. An important element in OS response is the Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) leucine zipper (zZip2) transcription factor, Nrf2 degradation, and localization controls the transcription of many OS-response genes. This analysis aims to map the Nrf2-mediated OS response pathways via the ToxCast database, and related bioassy response patterns to chemical classes. ToxCast phase II contains high throughput toxicity data for 1,865 commercial
chemicals, pesticides, and pharmaceuticals. The effects of these compounds on gene and protein expression in multiple cell lines are evaluated via 700 biosassays. The unprecedented collection of toxicological data is ideal for in silico elucidation of toxicity mechanisms and AOPs. We identified all ToxCast assays pertaining to the Nrf2 OS-response pathway and evaluated the relationships between electrochemical parameters and Nrf2 response pathway, as evaluated by ToxCast. Several iZip transcription factors exhibit patterns of expression similar to Nrf2. Furthermore, the toxicity of chemicals across multiple iZip factors tends to lie outside the region of general cytotoxicity and burst effects. The patterns of pathway-specific gene expressions observed in ToxCast and the relationship with reactivity and shape parameters will help unravel the diverse network. The Molecular Design Research Network (MODRN) aims to use the property-effect relationships and advanced modeling and machine learning techniques to create design guidelines for minimizing oxidative stress effects generate by commercial chemicals.

WE320
Comparison of Ready Biodegradability Estimation Methods for Fragrance Materials and Application to Musk
R.S. Boething, Exposure Assessment Branch US EPA Office of Pollution Prevention and Toxics
Biodegradability is fundamental to the assessment of environmental exposure and risk from organic chemicals. Predictive models can be used to pursue chemical design (green chemistry) objectives, which are most effectively met when models are easy to use and available free of charge. The Institute of this work was to evaluate no-cost estimation programs with respect to prediction of ready biodegradability. Fragrance materials, which are structurally diverse and have significant exposure potential, were used for this purpose. Using a database of 222 fragrance compounds with measured ready biodegradability, 10 models were compared on the basis of overall accuracy, sensitivity, specificity, and Matthews correlation coefficient (MCC). The 10 models were VEGA® Non-Interactive Client, START (ToxTree®), Biowin®1–6, and two models based on inductive machine learning. Applicability domain (AD) was also considered. Overall accuracy was ca. 70% and varied little over all models, but sensitivity, specificity and MCC, showed wider variation. Based on MCC, the best models for fragrance compounds were Biowin6, VEGA and Biowin3. VEGA performance was slightly better for the compounds it identified as having “high reliability” predictions (AD index >0.8). However, removing compounds with one and only one quarantine carbon yielded a similar improvement in predictivity for VEGA and Biowin 3/6, but with a smaller penalty in reduced coverage. Results from the models were then used to compare musks across the various structural classes and make inferences about their environmental attributes.

WE321
Substitution of long chain fluorinated copolymers for durable water repellent (DWR) textile modification
S. Schellenberger, Department of Applied Environmental Science ITM; H. Arrold et al., Research Institute Ltd / Department of Chemical and Biological Engineering; I. van der Veen, VU University Amsterdam / Institute for Environmental Studies
Fabric’s repellency against polar and non-polar liquids while maintaining breathability is a key functionality in modern textiles. Durable water repellent (DWR) polymers can provide these properties and are therefore produced in high tonnages. Despite differences in polymer architecture that mainly depend on end-uses in textiles, all state of the art DWR polymers have hydrophobic side chains linked to a polymer backbone. These non-polar side chains are based on hydrocarbons, silicones or perfluorinated (PF) moieties and need to be closely packed and orientated towards the fiber surface to achieve an “umbrella-like” repellent effect. Textiles treated with these polymers however might be a source of leaching chemicals used by degradation of the polymeric (non-fluorinated) backbone or the release of impurities (from production) during the life cycle of textiles. Considering worst case scenarios, degradation can result in persistent contaminants which have been found in textiles already. The phase-out of persistent, bioaccumulative and toxic long chain PF materials that were used for very effective DWRs in the past resulted in the development of alternatives based on short chain fluorocarbons (C6 and C4). Although these side chain fluorinated polymers can be considered less toxic and less bioaccumulative, their potential to form mobile and persistent contaminants is not fully understood. Other “eco-friendly” DWR technologies based on silicones or novel star-shaped hydrocarbons (dendrimers) have unclear degradation pathways as well. This lack of resolution in understanding corresponding risk can be attributed to the interdisciplinary collaboration SUPFES (http://www.supfes.eu) is to help industry “ecofriendly” DWR technologies based on silicones or novel star-shaped hydrocarbons (dendrimers) have unclear degradation pathways as well. This lack of resolution in understanding corresponding risk can be attributed to the interdisciplinary collaboration SUPFES (http://www.supfes.eu/) is to help industry.

WE322
Aquatic toxicity of biofuels - Using ecotoxicological methods for sustainable biofuel development
S. Heger, Institute for Environmental Research, RWTH / Institute for Environmental Research; K. Bluhm, Instit.for Environnement.Research, RWTH Aachen Univ. / Institute for Environmental Research; N. Anders, RWTH Aachen University / Enzyme Process Technology; A. Gers, RWTH Aachen University / Department of Environmental Social and Spatial Change; T. Seiler, RWTH Aachen University / Ecosystem Analysis; A. Scheiffer, H. Hollett, RWTH Aachen University / Institute for Environmental Research
The increasing global demand for energy, e.g., for the fossil fuel-dominated transport sector requires the development of alternative energy sources. Due to declining production capacities and environmental concerns of fossil fuels, such as emissions of greenhouse gases, governmental support for the development of renewable energy sources, such as biomass-derived fuels, increased considerably. A rise in biofuel production and consumption increases the risk of a release into the environment. The lack of ecotoxicological data on biofuels impedes a sufficient assessment of their environmental hazard potentials. Ecotoxicological biotests can be applied in a prospective assessment of the hazard potential for aquatic ecosystems and allow an identification of the most environmentally friendly biofuel even at a very early stage of the development. This study focused on the investigation of one potential biomass-derived fuel candidate, 2-(2-chloroethyl)thiophene (2-CHT). The ecotoxicological investigation was conducted by means of acute and chronic Daphnia magna biotests according to OECD 202 und OECD 211 adapted to the testing of biofuels, as well as a Daphnia magna population assay. We used a toxico-kinetic-toxicodynamic (TKTD) model to describe the process leading to a toxic effect and subsequently integrated this model into an individual-based population model. This allows for the extrapolation of population level effects from individual-toxicity testing. The TKTD model is deemed suitable to describe effects resulting from time-variable exposure of the volatile substance. Results from ecotoxicological biotests were used to inform the process-based effect model. Model predictions at the population level were tested along independent population data. This evaluation reveals that the methods applied can be used in a sustainable biofuel development to assess the effects on a population level. However, testing of further potential biofuels has to be conducted. Acknowledgement: This work was performed as part of the Research Cluster "Tailor-made fuels from biomass", which is funded by the Excellence Initiative by the German federal and state governments to promote science and research at German universities.

Challenges of regional and global modelling nitrogen and phosphorus in agriculture supply chains (P)

WE323
Macroalgae production and biorefining in Denmark - a life cycle assessment
M. Csoka, Department of Knowledge Engineering, Eindhoven University of Technology; L. Pardon, UMR ENSAM / Institut National de Recherche Agronomique; J. Caliman, SMARTRI; R. Marichal, B. Dubos, J.-P. Bani, University / Institute for Environmental Research; N. Anders, RWTH Aachen University
Macroalgae is a key biomass for the future development of a biobased society. A model of the brown algae Saccharina latissima is here utilized as feedstock for a bioenergy system which produces bioethanol, proteins and fertilizers. A Life Cycle Assessment was conducted from cradle to grave; i.e. from production to end-use in textiles, all state of the art DWR polymers have hydrophobic side chains linked to a polymer backbone. These non-polar side chains are based on hydrocarbons, silicones or perfluorinated (PF) moieties and need to be closely packed and orientated towards the fiber surface to achieve an “umbrella-like” repellent effect. Textiles treated with these polymers however might be a source of leaching chemicals used by degradation of the polymeric (non-fluorinated) backbone or the release of impurities (from production) during the life cycle of textiles. Considering worst case scenarios, degradation can result in persistent contaminants which have been found in textiles already. The phase-out of persistent, bioaccumulative and toxic long chain PF materials that were used for very effective DWRs in the past resulted in the development of alternatives based on short chain fluorocarbons (C6 and C4). Although these side chain fluorinated polymers can be considered less toxic and less bioaccumulative, their potential to form mobile and persistent contaminants is not fully understood. Other “eco-friendly” DWR technologies based on silicones or novel star-shaped hydrocarbons (dendrimers) have unclear degradation pathways as well. This lack of resolution in understanding corresponding risk can be attributed to the interdisciplinary collaboration SUPFES (http://www.supfes.eu) is to help industry.
agro-ecosystems. We identified various operational and process-based models that could be applied to oil palm, although most of them were not specifically adapted for tropical perennial crops. To our knowledge, APSIM is the only available process-based model of oil palm cultivation that includes N losses [7], but the accuracy of its N loss estimates was untested. Other means of assessment were based on statistical models [8]–[10] or other approaches [11]–[13]. Nitrogen balance models reveal important uncertainties (Figure). Estimates of N0 and NO1 rely on very uncertain and sensitive parameters such as the clay content for instance with the SQCB-NO3 model [13]. These uncertainties are due to a lack of understanding of processes. In particular, it is difficult to study and understand N dynamics over the whole lifespan of oil palm crops (20-25 years) and to account for varying agricultural practices. Adequate data to characterise various cropping systems is often lacking. Further research is required to develop a procedure that provides a robust assessment of the environmental impact of N management in oil palm cultivation. Particularly, this procedure should have the capacity to account for a range of soils, climates, and management practices over the whole cycle. One track to be pursued is the development of an agro-ecological indicator based on Indigo concept [14], [15]. Such an indicator could help to reduce uncertainties in LCA of tropical perennial crops.

WE325
Quantifying within-farm nitrogen and phosphorus efficiency in grazing-based dairy production systems
C. Gourley, S. Aarons, Department of Environment and primary Industries / Soil Science; R. Eckard, The University of Melbourne / Faculty of Veterinary and Agricultural Sciences
The ongoing trend for increased intensification is occurring in grazing-based, as well as confinement-based dairy farms, worldwide. Grazing-based dairy farms are increasingly reliant on imported feed and fertiliser, with consequent greater nitrogen (N) and phosphorus (P) flows, transformations and losses, and a decreasing dependence on N inputs from N fixation by pasture legumes. While it is important to understand how much N and P is imported and subsequently removed in product on dairy farms, the ability to quantify within-farm flows and transformations, as well as the spatial and temporal distribution of N and P, is essential to identify opportunities for improved management. The purpose of this paper is to outline key components of N and P flows and transformations within grazing-based dairy operations, and propose simple metrics which can be used to quantify N and P use efficiencies and manure management practices, using readily collected information from dairy farms. The linking of farm-gate balances with internal N and P cycling processes provides opportunities for targeted improvements in N and P capture and recycling by farmers, and reducing losses of N and P from grazed dairy systems. In particular, this should include: (i) quantifying key nutrient inputs, outputs and stores (i.e. feed, manure) and nutrient balances and efficiencies at a simple (farm-gate) level, (ii) quantifying grazing animal N and P intakes and N and P use efficiencies, at least within each season, (iii) quantifying N and P distribution and loading rates at a paddock management scale, including inputs and outputs such as fertiliser applications, forage removal, and importantly grazing animal excreted N and P, and (iv) quantifying the amount and proportion of excreta N and P deposited in unproductive areas which go uncollected, as well as the amount and proportion of excreta N and P collected and redistributed. Such information from suitably comparable farms, can be used to benchmark farms across a range of indices and target farm management improvements. These data targeting within farm spatial and temporal variability need to be factored into LCA studies conducted on grazing based dairy farm systems to more effectively describe the effectiveness of N and P recycling and loss.

Prospective Life Cycle Thinking approaches for the definition and implementation of sustainability strategies in industry and policy making (P)

WE326
Prospective LCA for the implementation of an innovating technology recovering nutrients from urine
E. Igoe, E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTB
The risk of phosphorous depletion and of ore quality decrease in the coming future and the increased energy requirements for nitrogen production make urine, where these compounds are highly concentrated, an interesting resource and call for efficient nutrients recovery process. The ValuefromUrine project (FP7 funding) aims at developing a new urine treatment technology based on an energy-efficient biore- electrochemical system. Life Cycle Assessment (LCA) is used to evaluate the overall environmental impacts and benefits of the technology, including its infrastructure and operations, the sewer network where treated urine is mixed with wastewater from other sources and the effects on wastewater treatment plants. The performance of the technology is expected to evolve during the project duration, from the lab tests to a prototype demonstrating its functioning. This emerging technology could be even implemented at larger scale in the market. As a result, life cycle inventory data can be largely affected by these scale changes, both at the foreground and background levels, and by technology evolution in the supply chain. The intended approach to tackle this issue is the definition of prospective scenarios based on Formative Scenario Analysis (FSA). First, the system is modelled to calculate the inventory data based on parameters, which can be either fixed (e.g. chemical properties) or variable (e.g. urine flow); and related to design choices (e.g. size of the cell components) or uncontrolled by the operator (urine composition). They constitute the technological impact variables (system element influencing the system behaviour, and are thus determined by the system choices) and the environmental impact variables for the FSA. The relations and effects between impact variables are assessed in an impact matrix to identify the key variables. The future state of each key variable is estimated (including uncertainty distributions whenever possible) according to three scenarios: low, medium and large deployment of the technology (e.g. the more the technology is deployed, the larger is the scale, the more efficient it should be). Inventory data can finally be modelled for the different scenarios, allowing the calculation of the life cycle environmental impacts. The latter are represented through uncertainty distributions for the different deployment scenarios and are compared to the scenario without the technology (urine is directly sent to sewer network without pre-treatment).

WE327
Energy Recovery from wastewater Treatment Plants in Scotland: A Life cycle Analysis Approach
N. Amponsah; M. Trolldborg, I. Alders, M. Nijnik, R. Hough, The James Hutton Institute
Waste streams with calorific or resource value exist across all sectors of industry. Currently, many of these streams go to sewer or landfill. There is a vast potential to use such waste streams for renewable energy generation, especially within the water industry. The entire UK Water Industry uses a total of 7,703 GWh/year in electricity whilst Scottish Water currently consumes around 450 GWh/year of electricity. Energy used on clean water production is predicted to decrease in regulation; however, energy required for water supply and treatment. Problems such as leaking and inefficient water distribution, whilst wastewater treatment can potentially show a significant increase due to water quality drivers. Energy recovery strategies could help offset the electricity consumption of the wastewater sector and represent possible areas for sustainable energy policy implementation. In Scotland, many diverse strategies are in place to improve the quality of wastewater treatment, but unfortunately the more advanced the treatment, usually the more energy is required in order to produce better effluent quality. This necessitates a robust energy reduction strategy for the sector, but attaining the required level of water quality and energy savings can be associated with other environmental costs such as emissions from energy production and increase in runoff from sludge application to land. The goal of this study is to investigate both upstream and downstream opportunities for energy recovery or offsetting energy demand and report on potential energy savings and recovery from wastewater treatment plants in Scotland with its associated costs. Preliminary investigations suggest that the application of sludge to derelict lands for dedicated energy crops production, which as biogas can generate electricity and heat is a potential route for energy recovery. The project uses data from surveys of treatment plants, from site visits to selected facilities, from equipment suppliers, and from published technical literature to analyse the potential for energy recovery. Life Cycle Assessment (LCA) has been the analytical tool used to evaluate environmental loadings. Initial results from the analysis of the selected systems will be presented and analytic issues will be discussed, including an approach to derive budgets of water and energy inputs for water and waste processing, P stocks and flows. The project will be extended to evaluate implications of projections of population, energy and water demand for future energy costs associated with water supply and treatment.

WE328
From waste to product: a paradigm shift in Life Cycle Assessment applied to wastewater sludge
M. Pradet, Irstea Clermont Ferrand / Ecotechnologies; L. Aissani; J. Villot, Ecole des mines de Saint-Etienne - Institut Fayol / UMR CNRS EVS, J. Baudez, Irspe, TSCI research unit; V. Laforest, Ecole des mines de Saint-Etienne - Institut Fayol / UMR CNRS EVS
Since the last 20 years, wastewater treatment plants are become more effective in releasing a good quality water in the streams, according to stringent European directives aiming at maintaining and improving the streams environment (Directive 91/271/EEC, Directive 2000/60/EC). As a consequence, huge amount of wastewater sludge are produced each year. This sludge is currently considered as a waste but new industrial practices and European regulation (End-of-Waste directive) are ongoing to consider sludge as a valuable product. In Life Cycle Assessment (LCA), boundaries are well drawn up between life cycle foreground and background levels, and by technology evolution in the supply chain. The intended approach to tackle this issue is the definition of prospective scenarios based on Formative Scenario Analysis (FSA). First, the system is modelled to calculate the inventory data based on parameters, which can be either fixed (e.g. chemical properties) or variable (e.g. urine flow); and related to design choices (e.g. size of the cell components) or uncontrolled by the operator (urine composition). They constitute the technological impact variables (system element influencing the system behaviour, and are thus determined by the system choices) and the environmental impact variables for the FSA. The relations and effects between impact variables are assessed in an impact matrix to identify the key variables. The future state of each key variable is estimated (including uncertainty distributions whenever possible) according to three scenarios: low, medium and large deployment of the technology (e.g. the more the technology is deployed, the larger is the scale, the more efficient it should be). Inventory data can finally be modelled for the different scenarios, allowing the calculation of the life cycle environmental impacts. The latter are represented through uncertainty distributions for the different deployment scenarios and are compared to the scenario without the technology (urine is directly sent to sewer network without pre-treatment).
Life cycle assessment of biobased chemical building blocks made from European waste streams

C. Strazza, University of Genova / DICCA; F. Magrassi, University of Genova / DICCA; A. Del Borghi, University of Genova; C. Strazza, University of Genova, Polytechnic School / DICCA; M. Robba, university of Genova / DICCA

Reduction of energy consumption and local energy from renewable sources in the building sector constitute important policy measures needed to reduce EU energy dependency and greenhouse gas (GHG) emissions. The recent 2010/31/EU directive requires all member states to set minimum energy performance requirements for new buildings occupied and owned by public authorities. It also introduces the concept of cost optimality, requesting that minimum energy performance requirements are set “with a view to achieving cost-optimal levels”.

Member states shall establish a long-term strategy for mobilising investment also for private buildings, where the role for the private sector can be to invest in co-financing at instrument level and project level, to identify and develop projects at regional and local level, and to be creative in facing these premises and perspectives. (In response to prior requests...). On the basis of the data collected amongst partners of the ReNEW network, LCA of the different processes on development are realized (lab scale or pilot scale), and the results are compared to the impacts of the usual routes currently used to produce the same components. Data are processed in SimaPro software with EcoInvent database, and analyzed with the ReCiPe method.
is used in order to test the application of the tool for a real case study, with consideration of different prospective scenarios for energy demand, cost of gas and electricity.

WE333

When economy and environment meet LCA and cost effectiveness analysis of LED light engine for art illumination

G. benveniste, IREC; J. Llobetas, M. Perálvarez, J. Higuera, Catalonia Institute for Energy ResearchIREC; K.A. Cruz, Rutgers University & UMDNJ / Joint Graduate Program in Toxicology, Department of Biochemistry & Microbiology; J. Carreras, Catalonia Institute for Energy ResearchIREC

This study presents the environmental and economic analysis (Life Cycle Assessment - LCA and economic effectiveness assessment) of a fully-tunable LED based light-engine specifically focused on art-work illumination designed and manufactured in the framework of FP7 HI-LED project. When considering different criteria to boost the public acceptance of new technologies, LCA tool is not bringing enough information to address and orient changes in the use of LED lights and therefore a cost effectiveness study, taking into account either capital and operational costs of the LED luminaire has been performed throughout its lifespan. These two approaches offer additional information on the LED luminaire proposed and complete the technology profile description considering two aspects that may drive the change to adopt these products in day life. As a first step a conventional attributional “cradle to grave” LCA comparative analysis of the LED luminaire was performed comparing this light engine to an equivalent halogen luminaire used for art illumination. The comparison has allowed the detection of the environmental hotspots of the LED luminaire with respect to an equivalent halogen luminaire. The focuses on the LED engine requirements to illuminate frescos under low responsibility materials to keep the illumination below 200 lx. In this case, the colour perception can be largely influenced by the adaptation level. For this reason, the functional unit chosen responds to light an area of 4 square meters when installed at a height of 2.5 meters and obtaining a light intensity of 1600 lumens during 50000 hours in a wide range of colour temperatures. In order to satisfy the functional unit requirements, an appropriate number of LED and halogen lamps have been defined. The functional unit set the initial parameters to calculate the cost effectiveness of the LED technology, that are completed using the cost of the initial investment, the estimation of the electricity cost and their variation during the operational life of the luminaire, among others, that will enable the assessment of the break-even point for the LED technology. The results from both analyses are focused on improving LED systems design for optimal production, minimum resource consumption and decreasing the payback compared to conventional equivalent halogen luminaire.

WE334

Illustrating the Sistine Chapel: environmental economic costs accounting for new Solid State Light system

G. benveniste, IREC; J. Llobetas, Catalonia Institute for Energy ResearchIREC; K.A. Cruz, Rutgers University & UMDNJ / Joint Graduate Program in Toxicology, Department of Biochemistry & Microbiology; J. Carreras, Catalonia Institute for Energy ResearchIREC

Within the FP7 Led4Art project, a new lighting system based on LED technology has been designed and installed in the Sistine Chapel, at the City of Vatican, of improving its illumination quality. To do so, the new SSL (solid state lighting) system increases the illuminance levels on the frescos, preserves the paintings from heat and undesired radiations and enhances the visualization thanks to the higher CRI. The system required 45 hours of installation, providing 50000 hours of use in a wide range of colour temperatures. In order to satisfy the functional unit requirements, an appropriate number of LED and halogen lamps have been defined. The functional unit set the initial parameters to calculate the cost effectiveness of the LED technology, that are completed using the cost of the initial investment, the estimation of the electricity cost and their variation during the operational life of the luminaire, among others, that will enable the assessment of the break-even point for the LED technology. The results from both analyses are focused on improving LED systems design for optimal production, minimum resource consumption and decreasing the payback compared to conventional equivalent halogen luminaire.

WE335

From evaluation to design support: a proposal to incorporate social sustainability goals in building design

I. Cuerda, Technical University of Madrid / Department of Construction and Technology in Architecture; J. Neila, Technical University of Madrid / Construction and Technology in Architecture

This study deals with the Life Cycle Thinking (LCT) into the design of buildings and search for the identification of goals that architects should pursue to enhance the social performance of buildings. To achieve these aims a proposal on how modify the building design process with a LCT approach, a selection of aspects for the assessment of the sustainability of buildings and its translation into social goals has been made. The assessment of Environmental Life Cycle of products and processes has been used to help reducing the environmental impact. This approach is being very useful when it is applied to processes used to obtain always the same product and this is not the case in building design. Each building is a prototype and that makes more difficult to see how changes in the design process will change the assessment results. This is even more challenging when we refer to social sustainability where there is a double indeterminacy, first the cause-effect chain of the process and second the social impact pathways. Design buildings taking into account the LCT means to take design decisions thinking not only about the first day of the building in use but also about things like: What will happen when technical systems require changes to be updated? Is the building adaptable so that it can change its use without major renovation works? How are we going to deal with the end of life of the building? Is it design in a way that all some materials/products/systems could be reused or recycle? In recent years projects and labels defining how sustainability in the construction should be assessed were developed. The results of these works were the basis for the development of these social goals. First a review of the aspects assessed was made. The review allowed identifying the main common aspects. The next stage was the selection of the aspects in accordance with two criteria: the first one is that the aspects are affected by the design decision, and the second one that the requirement, valued throughout the aspect, go beyond the requirements established in the Spanish national regulation about construction works. The design and production of buildings requires an adaptation of the results of LC assessments in order to make them useful for the architects. The use of LCT requires a change in the way architects think about buildings. It is feasible to translate sustainability assessment of buildings into social goals more useful to the architects.

WE336

Allocation solutions for re-use and recycling flows: a case study of an insulating panel from waste tires recycling

A. De Luca, Politecnico di Milano; A. Valetti, G. Dotelli, Politecnico di Milano / Chemistry Materials and Chemical Engineering G.Natta

Defining system boundaries related to re-use and re-cycle flows is a debated topic in the international LCA community and different allocation solutions have been proposed so far; in particular, in open-loop systems (recycled material from one product system to a different product system) there is much uncertainty about which life cycle assessment results can change its use without major renovation works? How are we going to deal with the end of life of the building? Is it design in a way that all some materials/products/systems could be reused or recycle? In recent years projects and labels defining how sustainability in the construction should be assessed were developed. The results of these works were the basis for the development of these social goals. First a review of the aspects assessed was made. The review allowed identifying the main common aspects. The next stage was the selection of the aspects in accordance with two criteria: the first one is that the aspects are affected by the design decision, and the second one that the requirement, valued throughout the aspect, go beyond the requirements established in the Spanish national regulation about construction works. The design and production of buildings requires an adaptation of the results of LC assessments in order to make them useful for the architects. The use of LCT requires a change in the way architects think about buildings. It is feasible to translate sustainability assessment of buildings into social goals more useful to the architects.

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WE338

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further and to use LCA to define and quantify environmental improvement objective for all its plants. For this purpose, two generic models able to realize LCA of all Knauf Insulation plants in Europe have been realized, one by product types (glass and rock). These models are designed to be used for Ecodesign but also for EPD. The model for glass mineral wool is described in the following paragraphs. The model, implemented in GaBi 6, is built in a similar pathway than the producing of a product: a good understanding of the different workflows and the data collection. The model is made of five generic components, includes, for each step, all the raw materials that can be used in one of the factories as well as all the energy sources. Parameters allow to define the amount of each raw material consumed, therefore the model can be adapted to any factory simply by setting these parameters accordingly. Moreover, all elements than can be different from one plant to another can also be specified by parameter, for example, transport distance or energy source. The model can also be adapted to almost all Knauf Insulation products by using parameters where necessary: for example, several products have different binder contents, so a parameter defines the amount of binder. As some materials can be recycled at several stages of the process, special attention has been paid to recycling loops inside the model. The high flexibility of the model allow its used as Eco-Design tool or to realize EPD.


Major international events, such as EXPOS and Olympic Games, may have non-negligible impacts on the environment. Most of the structures built for these events have generally a useful service life limited to the duration of the event. Unlike traditional buildings, whose operational phase is very long and by far the most impacting one, construction and dismantling phases become much more relevant in terms of impact. This is even more true when the choice of the materials in order to reduce the overall environmental impacts of the structure and in particular to reduce the resource depletion and the waste production. The focus for these particular constructions, as also suggested by the “EXPO Guidelines for Sustainable Solutions”, must be the possibility of reusing or recycling the entire structure or the components at the end of the event. To achieve the goal it is essential to act on the design phase of the building. The priority must be to simplify the disassembling phase of the structures and to guarantee a second life of the components. The objective of our study is to assess the sustainability, through the LCA tool, of a temporary structure to support its designing phase. The particular case study involved is the Brazilian pavilion, which will be installed during the forthcoming EXPO 2015 that will be held in Milan from May to October 2015. The aim of the research is thus to evaluate how much the design of the building and the choice of the materials can influence the environmental performances of the structure. Different methodologies for the allocation of the impacts at the end-of-life are compared. Preliminary results confirm the importance of the design phase for this particular sector. The predilection of natural and recycled materials in the construction phase and the prevalence of the second life of the components can significantly reduce the impact of the temporary structure. The same approach can be adopted for new low-energy buildings, whose burden is usually shifted from the use phase to the production of the components. The possibility to follow the real flows of all the materials at the end of the useful life of the pavilion will guarantee the availability of primary data that will be used to validate the results obtained. The study can finally be considered as reference for future LCA studies of temporary structure in major international events.

WE339 A methodology for improving the economic and environmental management of the packaging of drugs: integrating LCA and LCCA I. Boschi-Prigola, Universidade San Jorge; M. Fano, San Jorge University / Facultad ciencias de la salud; F. COCA, Universidade San Jorge

In the second half of the twentieth century, the pharmaceutical industry designed a huge variety of new drugs that needed to be protected, identified and dosed to offer them in perfect conditions to users. To make it possible, the use of different types of containers has grown. During these years, the weight of the materials and superfluous package were changed as well as the design of primary packaging. From the obtaining of the raw materials to their treatment and disposal as waste, these containers are associated with an environmental impact using the life cycle. As the conventional LCA (Life Cycle Assessment) does not include any cost analysis, which is a major criterion in decision making, LCCA (Life Cycle Cost Assessment) is the integrated method used to determine the cost effectiveness of a new container. The aim of this work was to evaluate the cost effectiveness of two primary containers (blister and sachet) of Buprofen 600 mg - Cinfal throughout their life cycle integrating the economic variables with a conventional LCA. The pharmaceutical industry, distribution systems, users and authorized waste managers were evaluated and in the same functional unit and system boundaries were selected for environmental and economic approach. The input-output methodology analysis of costs facilitated the integration of LCA and LCCA. Results obtained from this design allow the identification of the associated impacts according to each stage of the life cycle of the studied containers, providing a comparative analysis between both environmental and economic impacts. In addition, this research provides a useful methodology that improves the economic and environmental management of the primary packaging of drugs.

WE340 Integration of material and energy performance of buildings: I= M+E D. Anink, WE Adviseurs; E. Alsenas, Utrecht University; A. Meijer, A. Straub, Delft University of Technology / Faculty of Architecture and the Built Environment; G. Donze, WE Adviseurs

In the Netherlands and other European countries, the introduction of an energy improvements requirement in the building regulations has resulted in big improvements in the energy efficiency of new buildings. Now that we are going towards zero energy buildings as the new standard, the role of building materials and embodied energy on CO₂ is even more and more important. However, a broader assessment of the environmental impact of buildings, including the building materials, is not so commonplace yet. Since 2014, it is in the Netherlands obligatory to make also a so-called “material performance” calculation for new building projects (dwellings and offices). This new legal requirement raises the problem however, that for designers, project developers and property managers aiming for a sustainable building, it is difficult to look for optimal solutions because they have work with two separate indicators, one for energy performance and one for material performance. As a matter of fact, energy saving measures will have often a negative effect on the material performance because of the additional materials needed for these measures. This effect is very clear for instance in case of photovoltaic panels. To solve this problem, a new framework has been developed to integrate material and energy performances of buildings into a single indicator, based on LCA, taking into account not only the embodied and operational energy, but also other environmental impacts that are usually assessed in LCA. The LCA results of operational energy consumption in the building are calculated and combined with the LCA results of the building materials into a single aggregated impact score: I = M + E. Several choices and assumptions were made to fully integrate energy and material performances in an LCA approach. The system boundaries of the material and energy performance calculations needed to be defined and the distribution of collective energy infrastructure has been addressed. This new evaluation method has been developed and will be tested in practice by a consortium including building owners and construction companies. We will discuss methodological choices involved in this evaluation method and present some practice-based results. With the new integrated environmental performance indicator, it is from now on possible to find optimal solutions for sustainable building from both energy and materials perspective.

WE341 Combining site-oriented and product-oriented approaches for an enhanced environmental assessment of industrial projects P. Larrey-Lassalle, IRSTEA Montpellier; E. Loiseau, UMR ITAP; P.C. Roux, Irstea / UMR ITAP ELSA; D. Salze, Ecole des Mines d'Alès; G. Junca, Ecole des Mines d'Alès; M. Lopez-Ferber, Ecole des Mines d'Alès; R.K. Rosenbaum, National Research Institute of Science and Technology for Environment and Agriculture - Irstea / UMR ITAP

No single approach can address all of the environmental issues raised by the implementation of industrial projects. Different methods should be used, either individually or in combination, according to the questions to be answered. In this paper, the convergence of site-oriented and product-oriented approaches proves to be a challenge for performing a comprehensive environmental assessment of industrial developments. The environmental impact assessment of new measures is performed with a procedural method used to evaluate potential positive and negative environmental impacts of a future project (site-perspective). As EIA is a framework for conducting assessments, the EIA practitioner is free to choose the analytical methods considered most relevant to assess the environmental impacts of a given industrial project. Hence, one of them could be Life Cycle Assessment (LCA). LCA is an analytical tool used to perform the environmental assessment of a product, service or process in order to identify possible improvements throughout its life cycle (product-perspective). Several authors offered comparisons between EIA and LCA and discussed their similarities and differences. Yet, rare are publications inferring what each approach could learn from the other. Identified EIA weaknesses likely to be implemented by LCA are: (i) a limited geographical scope, since EIA addresses only local impacts whereas the standard LCA methods could add a regional or global perspective, (ii) the exclusion of effects related to activities upstream and downstream in the supply chain, whereas a life cycle perspective could identify indirect effects and pollution transfers, and (iii) an incomplete assessment of alternative technologies, that could be well-tackled with an LCA approach. This study aims at developing an integrated framework for the use of LCA in the EIA procedure. It aims to complement and update earlier work in the field, where a framework was suggested for the integration of different environmental tools (including LCA) in Strategic Environmental Assessment procedure (SEA), which is a meso or macroscopic EIA applied to policies, plans and programs. It will be shown in which steps of the EIA procedure, for which environmental aspects, to what extent, and for which purpose LCA principles could be applied. Then the remaining bottlenecks and methodological issues that could arise in doing so will be exposed and the areas offering research opportunities for improvement will be discussed.
WE342 Assessing future societies with Life Cycle Perspective
Y. Arushanyan, CESC Centre for Sustainable Communications and Div Environmental strategies research; Moberg, E. Ekener-Petersen, L. Aguirre Borges, KTH Royal Institute of Technology / CESC Centre for Sustainable Communications and Div Environmental strategies research

In a joint project with the 3rd KTH in cooperation with ICT industry and municipalities developed five possible future scenarios for Swedish ICT society. The scenarios and their development are used to learn about possible futures and their potential environmental and social impacts, and to provide basis for planning. Life cycle thinking is crucial for the assessment of future scenarios, however the detailed level of LCA is not useful when assessing complex systems, such as a society. Thinking in lifecycle of the whole environment for the environmental and social assessment with a life cycle perspective was developed within the project. Within this development relevant aspects to be assessed and indicators to be used for assessment were defined as well as a process for assessment. The process of the methodological framework development consisted of iterative steps: a literature overview, discussions within the interdisciplinary assessment group, and workshops with experts from academia, industry and regulatory administrations. A number of challenges were faced while attempting to address the inherent uncertainty and data restrictions. The outcome of this work is a methodological framework to assess the environmental and social impacts of future scenarios. The framework is using life cycle thinking however differs from the traditional LCA trying to deal with a broad and complex object and scope of assessment and not. Interactions are produced due to the LCA calculation model itself. Unlike a traditional LCA this framework does not imply using precise data and modeling all related processes. Instead, more qualitative analysis is done. The learning process of developing the framework and performing the assessment has, as in LCA, been a major benefit and outcome. Key words: Future scenarios, Life cycle thinking, Environmental impacts, Social impacts

WE343 Guideline for conducting a suitable sensitivity analysis in life cycle assessment: considering correlations and interactions within the calculation model
w. wei, Istrea Clermont Ferrand; P. Larrey-Lassalle, IRSTEA Montpellier; T. Faure, LISC IRSTEA; N. Dumoulin, Istrea - Lisc; P.C. Roux, Istrea / UMR ITAP ELSA; J. Mathias, Istrea - Lisc

Sensitivity analysis (SA) studies the robustness of results and their sensitivity to data quality and associated assumptions or models. It highlights the most significant set of model parameters to determine whether data quality needs to be improved, and to enhance interpretation of results. SA has become an important tool for linking the number of parameters that have to be introduced in the uncertainty analysis for the last two decades. A significant number of sensitivity methods are available. Each method presents its own strengths and weaknesses. Here we propose a methodology for conducting a proper SA which takes into account the effects of two main issues in the LCA calculation process: (i) interactions within the LCA calculation model and (ii) correlations within life cycle Inventory (LCI) inputs and outputs. Interactions are produced due to the LCA calculation model itself, and due to explicit causality relations. Correlations describe the relationship between random variables and are involved in the LCA inputs. Three typical SA techniques are considered here. The first is the local SA that focuses on identifying the important factors, which is particularly well adapted for a high number of uncertain parameters. The second is the independent global SA analyses the variability of results due to the variations of the input parameters over the whole domain of uncertainty, together with interaction effect among parameters. However, having independent parameter is the necessary condition for applying independent global SA. To overcome this limit, we then consider a third approach, the dependent global SA that makes minor modifications to traditional Sobol indices, which takes into account the correlations within LCI data. This approach has never yet been done in LCA. Finally, we propose a guideline for choosing the appropriate SA method depending on the characteristics of the model and the goals of the study. Our results clearly show that the choice of sensitivity methods should be made according to the magnitude of uncertainty and the degree of correlation.

WE344 Engineering Sustainability: Choice of Unsaturated Polyester Resins for cc-GRP Pipe Systems
V. Vladimirov, HOBAS; M. Pazdro, REICHOLD AS

In today’s economy efforts are taken towards re-using of available resources and using of resources which are re-generable, thus reducing the impact on the environment. For the automotive industry this includes the use of renewable raw materials represents an essential task. The study evaluates the different choices of unsaturated polyester resins (UPR) for production of centrifugally-cast glass reinforced pipe (cc-GRP) systems. The environmental impacts of three types of resin were evaluated and compared. The resins are: UPR standard, UPR containing recycled PET material (rPET-UPR) and UPR containing bio-sourced material (Bio-UPR). The analysis focuses on comparing the variations in environmental indicators caused by resin selection for three increasingly complex product layers (Base plate of cc-GRP shaft, cc-GRP shaft and 1km cc-GRP Pipe-system). The study equally provides an insight onto R&D and LCA collaboration across the supply-chain. One of the main challenges in LCA today is using specific data from the suppliers instead of generic data. The paper indicates how LCA tools and established R&D processes can be employed to transfer LCA calculations across the supply-chain. The use of specific Bio-UPR resin selected for evaluation results in higher environmental indicators than for the standard product (i.e. “total renewable energy”, “net use of fresh water” or “depletion of abiotic resources”). On the other hand, the bio-based component is just one of many product components. Particularly, high share of styrene and propylene glycol (PG) tend to increase variations (i.e. CO2 eq emissions calculation for PG or styrene are twice as high as for the bio-component used in production). Knowing this, it is possible to design new bio-based resins having more favorable sustainability footprints than those in-use today. The use of rPET-UPR results in better environmental indicators for the final product, especially as the quantity of material increases (i.e. for 1km pipe system). Bio-UPR and rPET-UPR are alternative choices which are realistic in terms of costs and which ensure the required quality for the manufactured products. rPET-UPR can be used for production of complete pipe systems, with positive environmental indicators. Mechanical properties of Bio-UPR restrict its usability and use of this resin presents similarities with the debate regarding use of bio-diesel.

WE345 New tool, Life Cycle Risk Assessment (LCRA): a application to renewable energy pathways
M. Lichou, IRIAF / Gestion des Risques; P. Roussaux, Université de Foiets / IRIAF

The concept of “Life Cycle Thinking » is not taken into account in traditional risk analyses. To integrate this fundamental concept to the risk analysis methodology, we propose a new method called “Life Cycle Risk Assessment» (LCRA). Following the model of the LCA methodology, we made some adjustments to the risk analysis methodology. Each of the four steps of LCA has its counterpart in terms of LCRA. The variations between these two methodologies reside on two key steps: (i) the inventory and (ii) the impact assessment. However, the goal of our study is not to directly adapt the LCA model, but to propose a new method which better serves the analysis of risks associated with the use of renewables. We illustrate the LCRA method by a case study: comparison of the risks associated with the wind and photovoltaic energies. The interest to consider the life cycle in a risk analysis is to identify and quantify the transfers of risks towards the different stages of the life cycle. We show that raw material acquisition or dismantling can be much more dangerous than the use. It is then possible to check the principles of the sustainable development for the criterion “Health & Safety”. LCRA can be very useful in the context of social acceptance of technologies considered as dangerous. LCRA is an operational tool and applies to all life cycles.However, LCRA evaluates only the risk of death and it is difficult to have a good accuracy on the results. This method is not designed as a precise tool to assess the risks but is used to consider the entire life cycle in a risk analysis. LCRA is a perfect tool and raises some questions in terms of completeness of the inventory of dangerous situations.

WE346 Social LCA: a text mining analysis for critical review
G. Arcese, University of Roma Tre / Business Studies; M. Lucchetti, University of Roma Tre / Department of Business Studies; L. Massa, Università La Sapienza; c. valente, Ostfold Research

Social Life cycle Assessment (SLCA) is an assessment technique to evaluate potential positive and negative social impacts of product/service and production using a life cycle perspective, lacking of standard and code of practice. The guidelines from UNEP/SETAC (2009) and the recent handbook for products (Forrest et al., 2014) are the most used for its methodological deepening and consolidation. Hence, the present study intends to classify the variety of contributions on SLCA in order to trace the evolution of the theme and to define the main lines of research. The use of cluster analysis enables to map the main orientations present today in the literature. The methodology chosen to conduct a comparative analysis of the literature is the automatic text analysis. The computer program inspected 51 articles all strictly related to methodology, all SLCA published between 2006 and October 2014. The scientific works were collected on the main bibliographic database selecting all available articles containing ‘Social LCA’ in the title, the keywords, in the text and in the conclusions. Through TaLTaC2 Software several articles were investigated by statistical analyses comparing different lexicographic-textual profiles occurring in the corpus (Bolasco 2000). The variable ‘year of publication’ both positive and negative specificity were calculated showing the research topic that characterize each period. The analysis of lexical correspondences shows clusters of authors identified according to the similarity of specific words and expressions. Text expression provides an overview of different solutions currently used in social LCA studies. Therefore a
plurality of methodologies on impact and risk assessment are emerged in the analyzed articles. Preliminary results show the absence of agreement on key aspects such as functional unit, system boundary, bottom-up and top-down approach etc. threatening the consolidation of this methodology. Weak and strong points of this technique will be presented in a critical discussion. The conclusion from our study is that it is challenging to classify the approaches in social life cycle assessment context, but at the same time, the critical review will help to identify the key factors on which research should really focus.

**Keywords:** Social LCA, Automatic Text Analysis, Clustering, Life Cycle Approach

**WE347 Environmental assessment of a varied productive reality: the case of Grana Padano**

S. Corrado, Universita Cattolica / Institute of Agricultural and Environmental Chemistry; A. Stroppa, Grana Padano Protection Consortium; G. Piva, M. Trevisan, Universita Cattolica del Sacro Cuore / Institute of Agricultural and Environmental Chemistry

The sustainability of the agro-food system, main theme of EXPO2015, is an important challenge for producers all around the world. The Grana Padano Protection Consortium, important protagonist of the Italian gastronomic tradition, wants to be part of it. Therefore, the Consortium is approaching the theme of environmental sustainability of Grana Padano starting from the evaluation of its environmental performance. The Grana Padano Consortium covers a large territory in the Po Valley and groups a big number of productive realities: in 2013 it comprised 4,634 farmers, 132 dairies, 156 matures and 177 packagers. The geographical localisation, the dimension of the plants and other parameters influence management choices and result in a quite varied reality. In this context, the present study, carried out by Universita Cattolica del Sacro Cuore and following the criteria of Life Cycle Assessment (LCA) reported in ISO standards 14040:2006 and 14044:2006, aims at defining a benchmark for the environmental performance of the Grana Padano production in order to support different practices which are more suitable. The evaluation of the benchmark is based on the definition of a representative cheese supply chain actors sample, composed by milk and cheese producers, matures and packagers. The criteria for the sample definition are the plants geographical location, mainly significant for milk production influencing the cow ration, and the plants dimension. Comparison among different practices is done on the basis of the criteria chosen for the sampling. The assessment follows the approach from cradle to gate, from the milk production to the packaging of cheese. The functional unit of the study is one kilogram of Grana Padano - 9 months ageing. The LCA is based on primary data collected directly from the supply chain actors. The first results on the impact on climate change underline that milk production is the process that mostly affect the environmental performance of Grana Padano, accounting from 89% to 96% of total GHG. Particularly, methane emitted form ruminal and enteric fermentation and manure management is responsible for about 40% of the GHG emissions. The presence of a biogas plant, fed with manure, both at the dairy or at the farm, reduces largely the emissions of methane and nitrous oxide, with benefits for the impact on climate change. Further results, including other impact categories, will be presented at the conference.

**WE348 How the public transport can be green? - Cooperating analysis between Miskolc and Novi Sad**

K. Szita Toth, University of Miskolc / Institute of World and Regional Economics; R. Sándor Bodnár, Bay Zoltán Nonprofit Ltd. for Applied Research / Department of Environment Management and Logistics; I. Budak, University of Novi Sad, Faculty of Technical Sciences / Production engineering

One of the main research topics of Horizon 2020 aims on developing a resource efficient transport, i.e. how to decrease the environmental impact of public transport in the cities. Our paper’s goal is to analyse the recent impacts and situation related to public transport in the bigger towns, with population of 100,000 inhabitants and over. The applied methods for the analysis is LCA. In the first stage we determined the function unit, which was the 10 km distance travelled. The system boarder was extended from vehicle production to end of use. The analysis incorporated the tram, bus, electrical bus and local train traffic. Obtained results presents the environmental impact the process that mostly affect the environmental performance of Granda Padano, accounting from 89% to 96% of total GHG. Particularly, methane emitted from ruminal and enteric fermentation and manure management is responsible for about 40% of the GHG emissions. The presence of a biogas plant, fed with manure, both at the dairy or at the farm, reduces largely the emissions of methane and nitrous oxide, with benefits for the impact on climate change. Further results, including other impact categories, will be presented at the conference.

**WE349 Life Cycle Assessment for the Sustainability of Contaminated Site Remediation**

C.C. Zhang, Environmental Sciences; V. Yassinskyy, University of Houston / Clear Lake / Department of Environmental Sciences

A limited number of case studies have been conducted to date on the use of Life Cycle Assessment (LCA) for the evaluation of sustainability of contaminated soil and groundwater remediation. These studies have generally suggested the usability of LCA but also pointed to the methodological flaws and other issues related to the complex remediation systems which hamper the use of LCA by practitioners and for decision-makers to ultimately incorporate sustainable strategies into soil and groundwater remediation. We have employed openLCA software and ELCD to examine past, on-going, and alternative remediation scenarios on the Geneva Industries Superfund site, Houston, Texas, including remote landfill, protective cap, slurry wall, continuous pump-and-treat (P&T), pulsed P&T and monitored natural attenuation (MNA). LCA serves well as a retrospective as well as a prospective impact analysis tool for the comparison of sustainability among remediation scenarios and for potential future remediation. The burden of streamlined data suitable for remediation use and the lack of assessment for social and economic pillars implementation of sustainable remediation. The complexity of the systems which hamper the use of LCA by practitioners and for decision-makers to ultimately incorporate sustainable strategies into soil and groundwater remediation. For example, LCA results confirmed that remote landfill was more sustainable than local incineration but the slurry wall already in place was the least sustainable subsystem with its construction materials contributed over 98% in most impact categories. LCA results also imply that future remediation should adopt pulsed P&T scheme in lieu of continuous P&T, whereas MNA can replace P&T if a long-term 30-year remediation is permitted at the site. In line with several reported studies, we concluded that current LCA methodology tends to favor less invasive remedial options and overestimate the material and energy related secondary environmental impacts rather than the direct benefits of reduced primary environmental impact at the local level which is the true driver for the implementation of sustainable remediation. The burden of streamlined data suitable for remediation use and the lack of assessment for social and economic pillars essential to sustainable remediation are identified as the major impediments for the widespread acceptance of LCA in environmental remediation. General solutions with these shortcomings will be illustrated with our case study data and future research and development opportunities in sustainable remediation will be highlighted in this presentation.

**WE350 Comparative LCA of decentralized wastewater treatment alternatives for non-potable urban reuse**

T. Offer, E. Friedel, Technion - Israel Institute of Technology

Traditional approaches to water management have focused on modifying water storage and flow patterns. However, in a growing number of cases the available water is not sufficient to meet basic water needs. In urban areas often attainable water sources have been over-allocated and new water sources which can be cost-effectively developed are no longer available. Water conservation, which was viewed in the past as a temporary measure for times of drought or emergency water shortage, is nowadays seen as a main long-term strategy for increasing water availability for water utilities, environment and community. With many communities approaching the limits of their readily available water supplies, water reclamation and reuse has become an attractive option for conserving and extending available water sources. Municipal wastewater (WW) represents a reliable and significant source for reclaimed water, as it is consistently available, even during periods of drought. Conventional wastewater treatment plants are usually large, centralized facilities generally located in suburban areas worldwide, but the advantages of the centralized approach over decentralized ones are questionable. In this study we use Life Cycle Assessment to compare between the environmental impacts of four alternatives for a hypothetical city’s water-wastewater service system. One is the most common, centralized approach for WW treatment, in which WW is treated at a large wastewater treatment plant (WWTP) and is then discharged to the river. The three alternatives represent different scales of distribution of the WW treatment phase, along with urban domestic water reuse for non-potable uses (toilet flushing and landscaping). The first alternative includes centralized treatment at a WWTP, with part of the reclaimed WW supplied back to the urban consumers. The second and third alternatives implement de-centralized WW treatment with local reuse, one at cluster level (several multi-family buildings) and one at building level (40 households). Life cycle impact assessment results show a consistent disadvantage of the prevailing centralized approach under local conditions, consisting of seawater desalination as the marginal source of water supply. Relative scores of the other three alternatives vary across different impact categories. Contribution of the different systems to the total score is discussed and sensitivity analysis in regards to major model parameters, such as water source and electricity mix, is presented.

**WE351 Environmental performance assessment of football fields with synthetic grass matrix using natural coal for rubber filler from end of life tires recycling**

S. Sbaffoni, L. Cutai, ENEA

Rubber coming from recycling of end of life tires (ELT) can be used both as material for manufacturing new products and for energy recovery. Among the possible uses as material, recycled rubber can be used as filler in football fields with synthetic grass. In Italy there is a huge production of ELT, about 300.000 tons/yr, and about 70% is used for energy recovery. Nevertheless the use of ELT as material is increasing and about 10% of recovered ELT are used for sport fields and applications in the sport sector. The use of recycled rubber from ELT for football fields, despite its growth, is
subjected to a parallel growing in concerns for possible impacts coming from rubber or its components dispersion into the environment and for the health of the users. In order to try to understand main impacts coming from the above cited issues, the present work shows main results of a comparative analysis of environmental performances of synthetic grass football fields with filler in rubber from ELT or with organic filler. The evaluation has been made using LCA methodology according to ISO 14040 series. The functional unit defined for the study corresponds at one football field in synthetic grass for a total surface of 7,668 m² and systems analyzed are: RF (syntethic grass football field with rubber filler): deposit of ELT at the generation point, commination phase with the production of granulate rubber, granulate rubber on site, oil extraction, pretreatments, cracking for the production of light hydrocarbons, polymerisation, production of plastic granulate rubber, granulate rubber on site (mixing, rolling and packing) and its on-site installation; trasports from different phases are included. End of life has been considered, including the removal, transport and treatment of syntetic grass layer and filler for their treatment (landfilling and/or recycling). OF (syntethic grass football field with organic filler): as the system above but with filler organic material including production (if primary Raw material) and deposit of organic materials, and end of life treatment. Different performances of the two types of football fields have been taken into consideration concerning in particular the different needs for maintenance and the overall service life of the products (filler plus synthetic grass).

WE352 Life cycle assessment of vegetative, reflective and traditional roofs: A case study for the Lebanonese context M. El Bachawati, University of Balamand / Chemical engineering department; R. Mamleh, Chemical Engineering; T. Dandres, CIRAIG; C. Nassab, The United Nations Development Program; H. El Zakhem, University of Balamand; R. Belarbi, University of La Rochelle / Laboratory of Engineering Science for Environment LabESE.

Abstract A vegetative roof is a "roofing system that promotes the growth of plants on a rooftop". Vegetative roofs offer many advantages, such as an embellishment of the roofing system, an enhancement of water management, and a reduction in energy consumption and heat island effects. Lebanon, a country situated in the Middle East, imports 98% of its energy (non-renewables) and experiences flooding during heavy rains. The objective of this research is to determine if the installation of vegetative roofs is truly superior to traditional roofs for the Lebanese country. A life cycle assessment is performed, comparing an existing extensive green roof installed at the Lebanese Central Bank to the following types of roofs: extensive green, reflective and traditional gravel-asphalted. The functional unit used for the comparison is the implementation of a roofing system on a surface of 834 sqm and for 45 years". A life cycle inventory was established and modelled using the SimaPro software. Results indicated that, for the existing extensive green roof, the waterproof membrane and the substrate layer were the highest contributors to the potential environmental impacts. When comparing the four types of roofs, results showed that, for all impact categories, the extensive green roof had the less environmental impacts. Sensitivity and uncertainty analyses were also performed to check the robustness of the results. Future research is to be performed on the effect of different substrate composition. Research will also be done on the effect of vegetative roofs on water retention and reduction in energy consumption for the Lebanese country. Keywords: Life cycle assessment, green roofs.

WE353 Challenges in applying LCA at the research stage: case study on biotreatment of pollutants in drinking water resources L. Muñoz; 2–0 LCA consultants; E. de Vries, Bioclear; J. Wittenbol.

Research projects aiming to develop new technologies, products, and services increasingly use life cycle assessment (LCA) as a means of providing information about the potential for sustainability of the technology under development. While using LCA at this early stage clearly provides benefits in terms of steering decisions towards sustainable choices, this collides with the practicalities of LCA: properly scoping the study, collecting (the sometimes unexisting) data and dealing with the uncertainty of the results from assessing a technology that does not exist yet in the market. We give an overview of the main challenges in the early stages of LCA practitioners encounter in this kind of prospective assessment and how they can be overcome, with the example of an LCA of biotreatment of water resources, carried out for the EU-funded project BIOTREAT (www.biотreat.org). BIOTREAT aims at developing new technologies for bioremediation of drinking water resources contaminated with micropollutants such as pesticides and pharmaceuticals. The basis of the proposed technologies is the introduction of specific degrading microorganisms or microbial consortia into existing sand filters at waterworks. We focus on the following aspects: defining the scope (number of scenarios, target pollutants, geographic delimitation, system boundaries), data collection, type of modelling (attribute/consequential), uncertainty (limitations of using lab-scale data and expert judgement) and the integration of LCA results with a parallel economic assessment. In the case studied of polluted water in a waterworks is compared to two alternatives, namely granular activated carbon (GAC) adsorption, and re-location of abstraction wells. Besides the LCA, an economic assessment including financial costs is performed, and the two assessments were integrated using monetization of environmental impacts according to the Stepwise LCA method from 2–0 LCA consultants. We show that LCA, applied at the research stage, can provide useful information to technology developers, opening their eyes to a completely different perspective that goes beyond their work in the lab. It is our conclusion that the role of LCA in this context is rarely to provide strong claims, but rather to identify hotspots, and to provide a first quantitative impression of relative performance, where what matters are orders of magnitude and not decimal points.

WE354 Reflections on User Assertions about LCA Tools S. Bajaj, A. Datta, Federation of Indian Chambers of Commerce Industry / Quality Forum; s. gupta, Quality Forum

We develop economic decision-making paradigms that support users in understanding and interpreting environmental concerns and want of scientific knowledge and access to eco-efficient technologies limiting their choices, developing countries like India are in dire need of simple tools that can help evolve and evaluate locally relevant options to manage environmental impacts. They must chart their developmental trajectory in a way that does not import unsustainable consumption and production patterns or make more environmental disasters. Considering the total cost in terms of economic benefits and environmental fixes, many technologies that seem too expensive at the moment may after all be worth their initial cost. Life Cycle thinking with a combination of LCA and LCC tools seems to offer an intuitively appealing method for evolving and evaluating choices both at the national and business policy levels. Choices could include eco-innovations, life styles changes, changes in consumption patterns and working changes in production technologies and markets, changes through institutional adaptations, etc. With this background, the questions we address in this paper are: How can Life Cycle Thinking practices and tools help in the Indian context? What governmental and business needs can they meet? How should they be positioned and extended to deliver more value? The paper discusses findings from structured interactions over the past two years with business, government, and community on their assertions about usefulness of LCA tools. The assertions show that prospective users are looking for LCA studies that can help evolve more options for sustainable technologies suited to the local context. They also perceive value addition from LCA through improved understanding of upstream and downstream activities to better manage costs, risks, impacts, etc. In conclusion, we build upon their assertions to present directions for future development of LCA tools that can facilitate evolution and evaluation of options to fulfill some typical goals relevant for developing countries, e.g. global competitiveness, access to affordable energy, cleaner environment.

WE355 Approach for sustainability assessment guided by life cycle thinking - application to virgin olive oil production G. Busset, INP-ENSIACET; J. Belaud, Université de Toulouse INP-ENSIACET CNRS UMR 5503; M. Montreajad-Vignoles, Université de Toulouse / INPENSIACET LCA Laboratoire de Chimie Agrico Industriale INRA UMR CAI; C. Sablayrolle, INPENSIACET

Context & Objectives Nowadays, any strategic decision must be in accordance with the sustainability principles. Therefore, companies need to evaluate the consequences of their activities and products onto environment, economy and society. Life cycle assessment (LCA) from Life cycle thinking (LCT) is one of the major environmental impact evaluation methods for product, process or service and the trends is to integrate economic and social aspects to become a life cycle sustainability assessment (LCSA). However, LCSA is still in its infancy and needs methodological proposals to be applicable. In this context, the present study proposes a sustainability assessment of the French olive oil agro-industrial sector. Methodology In doing so, a systemic approach for decision making and based on the coupling between LCSA, enterprise modeling, chemical process modeling and multicriteria analysis is proposed. The approach relies on a formalization of system modeling for LCSA. It is an integrated process-product-enterprise (PPE) approach with a multicriteria analysis. It helps to unleash the following shackles: how to integrate social, environmental and economic aspects for sustainability assessment. Results The results showed that the approach is effectively applicable and relevant for sustainability assessment. The application to olive oil production has been a part of the project MFP-EU project (1). A large amount of data was collected and has permitted to build olive oil production models and enterprise realistic models. The results showed that the PPE approach is effectively applicable for sustainability assessment. Acknowledgement The authors would like to thank the Interreg Program SUDOE for the financial support of the OiLCA project. Reference (1) OiLCA, 2011. Mejora de la competitividad y reducción de la huella de carbono del sector del aceite de oliva mediante la optimización de la gestión de residuos e la implantación de una etiqueta. www.olica.eu

Fate, Effects and Risk Assessment of Metals: Regulatory Perspective (P)

WE356 A simple method to reduce the risk of cadmium exposure from consumption of Iceland scallops (Chlamys islandica) fished in Greenland L. Bach, Aarhus University (AU), Arctic Research Centre / Department of
Bioscience; C. Sonne, Aarhus University / Department of Arctic Environment; F.F. Riget, Aarhus University; R. Dietz, Aarhus University / Department of Arctic Environment; G. Asmund, Aarhus University / Department of Bioscience Arctic Research Centre

This study investigated the levels and organ distribution of the toxic heavy metal cadmium in scallops from unpolluted Greenlandic waters. The scallops had an average cadmium concentration of 2.93 ± 0.05 mg/kg in the whole soft tissues and no concentration dependent effect was found for gender or size (both p > 0.05). The kidney was the primary organ for cadmium accumulation with a mean of 226.2 ± 111.7 µg/g wet weight, and despite the small weight of the kidney, it appeared as the principal contributor of cadmium with 92 % of the total cadmium body burden. The cadmium concentrations in the total soft tissues far exceeded the EU-level of 1 µg/g wet weight for cadmium in bivalves. Based on this, selective evisceration of the cadmium-rich kidney and digestive gland during processing can be regarded as a reliable measure to be taken in order to reduce the cadmium content of scallops used for human consumption.

WE357 Conversion factors for heavy metals and PFAS for fish muscle, liver and whole body
S. Faxneld, Swedish Museum of Natural History / Environmental research and monitoring; S. Danielsson, Naturhistoriska Riksmuseet / Department of Contaminant Research; E. Nyberg, A. Bignert, Swedish Museum of Natural History

Environmental Quality Standards (EQSs) (Specific Quality Standards (QS) when EQSs are not available) for priority substances and other pollutants, have been suggested by EU to protect aquatic ecosystems and human beings from adverse impacts of chemical contaminants. Mercury (Hg) is often measured in muscle to ascertain compliance with tolerance limits for human consumption, but the EQSbiota is set to protect predators of fish and evaluates whole body prey fish concentrations. Lead (Pb) and cadmium (Cd) are often measured in liver, but also here QSbiota is set for whole body. PFOS is measured in liver, but the EQSbiota is set to protect human health and evaluates edible parts, muscle or muscle+skin. Thus, if we can assume a relationship between concentrations in various tissues in the same specimen it is imperative to find these relations to enable conversions between various tissues and whole fish, to make the EQS meaningful regardless in what tissue the concentrations are measured. Hence, the aim with this project were to calculate conversion factors between liver and muscle (for PFOS), muscle and whole fish (for Hg) and liver and whole fish (for Cd and Pb) and to establish alternative EQSs or QSs in the various tissues, that can offer at least the same level of protection as the EQSbiota or QSbiota set for each compound. For PFOS, herring and perch were taken at different locations in the Baltic Sea and perch was also taken from several lakes in Sweden. For metal analyses, perch from different lakes in Sweden was used. Samples from liver, muscle and whole fish were taken and concentrations were measured. Thereafter linear regression analyses were performed between the different matrices and the regression line equations were used for each compound in order to recalculate the existing EQSs or QSs. For Hg, the EQSbiota in whole fish is set at 20 ug/kg wet weight (ww), after recalibration the new derived EQS in muscle is 21 ug/kg ww. For Cd, the suggested QSbiota for whole fish is 0.16 ug/g ww and the new derived QS in liver is 6.6 ug/g ww. For Pb, the suggested QSbiota in whole fish is 1 ug/g ww and the new derived QS in liver is 0.30 ug/g ww. For PFOS the EQSbiota is 9.1 ng/g ww in muscle and the new derived EQSbiota in liver is 144 ng/g ww. When comparing concentrations at the different locations, all locations had Hg levels above the target level, while for Pb and Cd all locations were below the target level. For PFOS, one site exceeded the target level.

WE358 Tracking temporal trend breaks (abrupt changes) of anthropogenic change in Mussel Watch metal databases
C. Guirard, Spanish Institute of Oceanography; C. López-Galindo, S. Gregores, Instituto Español de Oceanografía; A. Hernández-del-Valle, Instituto Politecnico Nacional; J.M. Martín, Universidad Carlos III de Madrid; J.M. Benedicto, J.A. Campillo, Instituto Español de Oceanografía; M. Albertos, Instituto Español de Oceanografía / Centro Oceanográfico de Murcia

The potential for structural changes in time trend concentrations of mercury (Hg), lead (Pb), cadmium (Cd), zinc (Zn), and copper (Cu) in the Mediterranean mussel, Mytilus galloprovincialis, was examined in Mussel Watch (MW) databases of metal pollution at eighteen coastal stations over a decadal period (1992 to 2007) along the Spanish Mediterranean coasts. Simultaneously, by using two statistical methods based on the approach of Hotelling's T2 and on a recent hierarchical cluster analysis approaches (imported from econometrics), we found single and multiple trend breaks for Hg (28% of the stations), Cd (17%), and Pb (11%) within trends, in connection with anthropogenic and subtle natural environmental changes. Also called change point problems, if not accounted for, these could bias time trend investigations and interpretations. We calculated trend rate differences of 39% and 45% up to 1 order of magnitude from classical linear trend assessments. We discuss sampling, analytical, and environmental (both natural and anthropogenic) sources of data set variabilities, showing that in practice, the overall 16-year analytical performance could be as elevated as the yearly sampling reproducibility. We demonstrate that environmental time trend interpretations benefit from undertaking prior structural change analysis. After decades of Mussel Watch marine chemical pollution assessments these have proven extremely useful, although the occurrence of trend breaks directly affects the long-term marine environmental monitoring strategies. Our results suggest a broader concept to design monitoring programs in agreement with rapid global anthropogenic and environmental changes.

WE359 Mussels in the Gulf of Naples (Italy): from industrial production to sustainable fishery
R. Scudiero, Department of Biology; M.G. Esposito, University Federico II of Naples; F. Trinchella, University Federico II of Naples / Department of Biology; P. Creti, University of Salento

In the recent years, the decline of wild aquatic organisms has led to the expansion of aquaculture in Italy. Among the molluscs, the edible mussel Mytilus galloprovincialis is the most sold in the Southern Italy, and the animals come almost exclusively from aquaculture. The Gulf of Naples is a 15 km coastal area located in the Campania region, subject to pollution from highly populated urban centers, port activities, and large industrial complexes, including oil refineries. Nevertheless, nowadays many mussel farms have been arising in this area following the closure of many important industries (the steel plant ILVA, for example). We monitored the quality of the mussels M. galloprovincialis farmed in this area converted to more environmentally friendly practices, in terms of heavy metal contamination. The concentration of toxic heavy metals (Pb, Cd) was determined on edible tissues of M. galloprovincialis specimens collected at different aquaculture farms. On the same animals was also determined the amount of metallothionein, the most important biomarker of metal pollution. The collected data were compared with those obtained from animals belonging to natural banks located in the same area, from animals belonging to farms located in pristine areas and from animals relaying in metal free seawater, in order to establish public health risks associated with consuming mussels harvested in the Gulf of Naples. Results show that the Pb and Cd contents in mussels from the Gulf of Naples do not represent a risk to human health, even in the period of their maximum accumulation, that do not depend on the harvesting or farming area, but rather on the seasonality. Indeed, in all instances the levels of the two metals were lower than the limits established by the European Food Safety Authority (EFSA 2005), that are 1 ug/g wet tissue for Cd and 1.5 ug/g for Pb. Data further demonstrate that the metallothionein functions go beyond the metal detoxification, thus opening new scenarios for the role of this protein in invertebrates. Finally, the relaying of mussels before marketing could improve the animals stress conditions, but would seem to have a slight effect on metal excretion, at least for the relaying period considered in this work. Therefore, we may conclude that including, or not, conversion of industrial sites in areas devoted to aquaculture represents a real opportunity to increase the number of companies and employees in fisheries production.

WE360 Assessment of the sensitivity of European freshwater environments to chronic exposure to nickel
A. Peters, Wca Environment Ltd.; G. Merrington, Environment Agency; I.A. Wilson, WCA Environment Ltd.; K. Delbeke, European Copper Institute; P. Van Sprang, ARCHE

Given the move within Europe towards the regulation of the levels of some metals in surface waters on the basis of bioavailability it is important to understand the variations in metal sensitivity that may be exhibited across the range of water chemistries. Bioavailability based Environmental Quality Standards (EQS) have been proposed for several metals, including copper, in the UK under as Specific Quality Standards (QS) when EQSs are not available for priority substances and other pollutants, have been suggested by EU to protect aquatic ecosystems and human beings from adverse impacts of chemical contaminants. Mercury (Hg) is often measured in muscle to ascertain compliance with tolerance limits for human consumption, but the EQSbiota is set for whole body. PFOS is measured in liver, but the EQSbiota is set to protect predators of fish and evaluates whole body prey fish concentrations. Lead (Pb) and cadmium (Cd) are often measured in liver, but also here QSbiota is set for whole body. PFOS is measured in liver, but the EQSbiota is set to protect human health and evaluates edible parts, muscle or muscle+skin. Thus, if we can assume a relationship between concentrations in various tissues in the same specimen it is imperative to find these relations to enable conversions between various tissues and whole fish, to make the EQS meaningful regardless in what tissue the concentrations are measured. Hence, the aim with this project were to calculate conversion factors between liver and muscle (for PFOS), muscle and whole fish (for Hg) and liver and whole fish (for Cd and Pb) and to establish alternative EQSs or QSs in the various tissues, that can offer at least the same level of protection as the EQSbiota or QSbiota set for each compound. For PFOS, herring and perch were taken at different locations in the Baltic Sea and perch was also taken from several lakes in Sweden. For metal analyses, perch from different lakes in Sweden was used. Samples from liver, muscle and whole fish were taken and concentrations were measured. Thereafter linear regression analyses were performed between the different matrices and the regression line equations were used for each compound in order to recalculate the existing EQSs or QSs. For Hg, the EQSbiota in whole fish is set at 20 ug/kg wet weight (ww), after recalibration the new derived EQS in muscle is 21 ug/kg ww. For Cd, the suggested QSbiota for whole fish is 0.16 ug/g ww and the new derived QS in liver is 6.6 ug/g ww. For Pb, the suggested QSbiota in whole fish is 1 ug/g ww and the new derived QS in liver is 0.30 ug/g ww. For PFOS the EQSbiota is 9.1 ng/g ww in muscle and the new derived EQSbiota in liver is 144 ng/g ww. When comparing concentrations at the different locations, all locations had Hg levels above the target level, while for Pb and Cd all locations were below the target level. For PFOS, one site exceeded the target level.

WE361 Development and application of predictive bioavailability models for the derivation of safe thresholds for assessing chronic nickel toxicity in freshwater sediments
M. van Ghelewe, ARCHE; M.A. Nguyen, Lab of environment toxicology and sustainable fishery
Slag with up to 3% vanadium content have many uses e.g. in agriculture. Part of the slag vanadium is in bioavailable form and thus posing potential threat to soil fauna. From a risk assessment perspective more ecotoxicological information is required for vanadium both in soils and solutions. Chemistry of the vanadium is complex and involves a wide range of oxygenated species but in environmental solutions only two oxidation states are stable, +IV and +V, from which +V is considered more toxic. In this context, questions of metal-specific factors (V oxidation state), exposure related issues (soil and water pH, DOC, soil OM), biological factors (sensitivity of test species, aquatic vs. terrestrial), and slag quality (V content, bioavailable vs. non-bioavailable V) need to be addressed being the main focus of this study. Ecotoxicity of two V oxidation states, vanadyl sulphate VO2+(+IV) and metavanadate NaVO3 (+V) were investigated both in water, and with two test soils of different organic matter content and coarse mineral subsoil (OM 0.5%). The pH of the test soils and waters were adjusted to three levels common in Finnish environment. The standard toxicity tests were performed by using earthworm Aporrectodea caliginosa, and for comparison cladocerans (Daphnia magna), bacteria (Vibrio fischeri), and duckweed (Lemma minor). The median lethal concentrations for earthworm ranged from 87.0 to 232.2, and from 76.9 to 305.5 mg V/kg soil DW for NaVO3 and VO2+, respectively. The subsoil with high pH promoted the highest toxicity. Of the four slags studied, steel slag from Raase power factory was the most toxic for the earthworms. In aquatic toxicity testing D. magna was the most sensitive species with median lethal concentrations range from 0.08 -1.77 mg V/L, and from 0.05-1.24 mg V/L for NaVO3 and VO2+, respectively. EC50 values for Lemma minor were in the range from 0.04 to 10.9 mg V/L reflecting again the importance of bioavailability issues. Only minor toxicity was observed in the experiments performed with V. fischeri. In terms of V risk assessment our results increased ecotoxicological information regarding species sensitivity, importance of exposure conditions, V chemistry and complex interactions of factors given above.

WE364 Sustainable recycling industries in developing countries - State of the art recycling metal standards and certification schemes
S. Valdivia, UNEP / Secretariat ILCI; S. Valdivia, Pontificia Universidad Catolica del Peru
The world’s consumer class is growing at exponential rates especially in the emerging and rapidly growing economies and so is the global resources consumption and waste generation rates. Metals are essential materials used in all industrial sectors and their availability is under threat in the mid- and long-term due to increasing prices and scarcity index. Recycling of metals has become a crucial activity worldwide and is well spread also in developing countries to ensure the supply of these valuable metals. However, metal recycling practice especially in developing countries still lack of sufficient recognition from key local stakeholders (e.g. local and national Government and consumers), resources and supporting infra-structure which is leading to acute social impacts on concerned actors around the recycling activity and low metal recovery efficiency. The Sustainable Recycling Industries Initiative (SRI: www.sustainable-recycling.org) is an international effort which has the aim to build capacity for sustainable recycling in developing countries. It is mainly funded by the Swiss Government and implemented by the World Resources Forum jointly with the Swiss Institute for Materials Science & Technology (Empa) through three components: Global Roundtable for Sustainable Recycling (GGRS): a stakeholder consultation process will be setup to support the development of guidance principles for sustainable recycling standards (GP4SRS) of critical and valuable metals. Capacity Building: Local capacities of SMEs for sustainable recycling will be strengthened in Colombia, Egypt, Ghana, India, Peru and South Africa. Life Cycle Inventories: Data will be developed to assess environmental and social life cycle performance for industrial activities through the improvement of local and regional expertise in Brazil, India and South Africa. The focus of this paper will be on the GGRS to be integrated by international partners and regional or sectoral initiatives such as the Waste Electrical and Electronic Equipment Forum, The Aluminium Standard Initiative, The World Gold Council, The OECD, among others. As the basis for the development of GP4SRS, it was identified the need to have a clear picture on key stakeholders as well as the state of the art of metals standards development and recycling metal standards certification schemes. The conclusion of the paper is to provide an update on the GGRS setup and a landscape of current recycling metal standards and certification schemes as part of the SRI project.

Reflections on bird and mammal risk assessment: past, present and future (P)

WE365 Bird community monitoring in chlorpyrifos-treated wheat fields in UK - breeding success and behavioural aspects
If a plant protection product which could pose a risk to birds is applied, it is important to understand the ecology of the bird community present in the target area.
crop in the application period in order to conduct a proper risk assessment. Therefore the aim of this study was to monitor the bird populations in wheat fields in UK, and to estimate the possible effects of the use of chlorpyrifos (CP) against orange blossom midge. The methodology for observation of the bird community present in arable land was adapted following the landscape approach. The main challenge is to deal with the year-to-year change (rotation) of the cultivated crop on single fields. Furthermore, winter cereal fields are characterized by a lower abundance of breeding pairs and a lower diversity of species than other agricultural areas, e.g. orchard systems. As counterbalance, birds are more visible within the arable landscape. The study was conducted in 2013 and 2014 in Cambridgeshire, an area representative for wheat cultivation and the use of CP. The subjects of the study were birds making use of treated and untreated cereal fields. In order to find birds using the fields, systematic nest searches were conducted regularly, by two combined methods: rope-dragging and watching-back. To obtain information on communities of birds present in arable fields, systematic observations of the number and species were conducted from selected positions using binoculars. Additionally, in 2014 a selection of birds was radio-tagged to check for changes in habitat use following the CP application. In 2013 there were 94 nests from 18 different species discovered in the study fields and their close surroundings. Only Skylark and Yellow Wagtail nests (35) were actually found within the crop itself. In 2014 the nest search focused on these two species, and in total 75 and 31 nests of Skylark and Yellow Wagtail respectively were monitored for fade. The results of the nest monitoring allowed the comparison between proportions of successful and unsuccessful breeding attempts in both treated and untreated cereal fields, and allowed the calculation of the total number of birds. The objective of this study was to evaluate foraging strategies and thereby reducing the risk of exposure to the residues. The changes in their home range caused by insecticide application may also be assessed. Radio tracking of Skylarks and Yellow Wagtails performing before and after the application of CP, demonstrated how the birds adapted their foraging behaviour after the application, and allowed to assess the potential consequences of this change on the breeding success.

WE366 Radio-tracking of birds - versatility to assess effects and spatial aspects of foraging behaviour

B. Giessing, Tier3 Solutions GmbH; R. Dittrich, Tier3 Solutions GmbH / Wildlife Ecology; M.M. Benito, Wildlife Ecology; A.A. Fernandes, F. Sotti, Tier3 Solutions GmbH; S. Norman, Ridgeway Eco; N.N. Poleitka, Dow AgroSciences LLC / Field Exposure and Effects Department; C. Wolf, Tier3 Solutions GmbH

If plant protection products are applied which pose a risk to birds, it is important to quantify the exposure. Theoretically birds might be exposed by direct over-spraying or by oral exposure through foraging on arthropods or plant material after application. Whether these theoretical pathways happen depends on the habitat type, the insecticidal species discovered in the study fields and their close surroundings. The survival of the birds could be confirmed to dehusk, the proportion of seed dehusked during feeding and the level of contamination achieved during dehusking which can be used in risk assessment for pesticides. Using frequency of occurrence in crops and risk based criteria for exposure, all studies have been reviewed to identify if possible at least one focal species per feeding guild, per crop in the new registration zones for southern and central Europe. Some focal species repeatedly appeared across a wide range of arable or tree crops, but not both, demonstrating broad adaptation to these different crop structures. Many have widespread distributions e.g. 15 of the focal species have a distribution covering all agricultural regions of Europe (Northern, Central and Southern zones). Three species; common breeding, serin and tree sparrow are restricted to the Central and Southern zones while another 4 species, Sardinian and fan-tailed warbler, kestrel and short toed lark are essentially restricted to the Southern zone. The authors consider the focal species identified are suitable for risk assessment in Europe at the zonal level and for further refinement of exposure through studies, i.e. radio-tracking and/or diet analysis, if necessary.

WE368 Outbreak-based surveillance as tool for assessment of regulation of plant protection products - Example of the rodenticide Bromadiolone in France

T. Quintaine, ONCFS / Unite Sanitaire de la France; M. Coeurdassier, Université de Franche-Comté. The SAGIR network took charge of dead animals to diagnose and confirm the disease, from which 416 had been confirmed. The confirmed toxico-epidemiological investigations concerned 17 species of birds of prey and mammals, including endangered species as Red Kite. The evolution of the legislation was followed consequently by a decrease of the quantity of bromadiolone used for treatments. The number of confirmed or suspected cases reported followed this evolution to reach in 2011 only eight cases. This retrospective study is a unique example of complete registration of the birds dying of rodenticide toxicity. Moreover, it confirms the usefulness of outbreak based surveillance for regulation process, especially for the risk assessment in the context of a post-authorisation to place on market process.

WE369 Use of dehusking in plant protection product risk assessment in Europe

M. Hackett, ADAS UK Ltd / Cambridge Environmental Assessments; A. Lawrence, Cambridge Environmental Assessments

Use of seed dehusking as an exposure refinement option for birds and mammals is discussed in EU guidance documents and zonal reviews of risk assessment information. Various criteria are set in EFSA (2009) which must be fulfilled before using dehusking as a quantitative refinement option. However, the use of dehusking as a refinement option is not applied consistently by EFSA nor by member states. Research is available on different aspects of dehusking including species which are confirmed to dehusk, the proportion of seed dehusked during feeding and the level of seed residue reduction achieved during dehusking behaviour of different species. This literature review of the available research on dehusking and feeding behaviour of birds and mammals aims to: Discuss the regulatory situation at EU and national level with regards to acceptability of dehusking as refinement option Identify species for which dehusking is a suitable option for refinement Evaluate and identify seed and spray treatment scenarios where dehusking is relevant for risk assessment and Evaluate their potential in relation to different seed type Recommend suitable default values for proportion of seed dehusked and residue reduction achieved during dehusking which can be used in risk assessment for different species Identify species and scenarios for which further research is required.

WE370 Common vole (Microtus arvalis) Ecology and Management: Implications for Risk Assessment of Plant Protection Products

P. Manson, Cheminova AS European Regulatory Office / Global Regulatory Science; T. Fredericks, Monsanto Company / Agronomic Environmental Assessment Services; R. Barfknecht, E. Bonnerius, Bayer CropScience Aktiengesellschaft BCS AG/EnSaETX; T. Jacobs, Federal Research Centre for Cultivated Plants

The common vole (Microtus arvalis) is a small herbivorous mammal with a widespread European distribution. Appearing frequently in regulatory risk assessments for plant protection products in the EU, it is the representative small
herbivorous mammal. Due to the conservative nature of the risk assessment for this species, this scenario frequently fails the lower tier long-term risk assessment. This poster describes common vole population dynamics, habitat and food preferences, their pest status within the EU and their use as a focal species in EU risk assessments. A common vole’s primary habitat is grassland. Periodic population outbreaks and movement of common voles onto agricultural land (considered secondary habitats) across Europe has resulted in the common vole being acknowledged as an agricultural pest in some areas. Movements into secondary habitats occur when the carrying capacities of their primary grassland habitats are exceeded. When movement of common voles occurs early in the crop growing season, significant damage to the crop can result from direct consumption of the crop shoots and leaves. Indirect crop damage is also caused by nest construction and extensive burrowing activity in the field, affecting the development of crop root systems. This can lead to reduced crop yields and crop failure in some cases. Consequently, common vole population management controls are practiced in many countries to prevent common vole populations reaching damaging levels. Common voles have short gestation periods and a high fecundity level that ultimately leads to a high resilience to population disturbance. Population movements into cropped areas are driven by density dependent factors occurring in primary habitats. These specific characteristics of the common vole indicate that the protection level afforded through mammalian risk assessment could be modified without affecting the common vole ecosystem service to the food chain within modified agricultural habitats. For EU risk assessments, it is appropriate to review and modify as required, the small herbivorous mammal scenario. Alternatives including the use of alternative scenarios that realistic food intake rates and using derived assessment factors or a combination of all alternatives, when conducting EU risk assessments is appropriate. Pragmatic modifications to risk assessments are already permitted in certain EU member states. It is reasonable to apply similar approaches across the wider EU.

### WE371 Monitoring the exposure of non-target birds and mammals to anticoagulant rodenticides - a new regulatory tool

R.F. Shore, Centre for Ecology & Hydrology (NERC); P.A. Hennyx, Centre for Ecology and Hydrology; L.A. Walker, Centre for Ecology & Hydrology

In Britain, the use of second generation anticoagulant rodenticides (SGARs) in open areas is thought to present the greatest risk of exposure in non-target wildlife. Until recently, the most acutely toxic compounds (brodifacoum, flocoumafen, difethialone) have been restricted to indoor use; open area use has only been permitted for bromadiolone and difenacoum. However, recent regulatory review has determined that it is possible to distinguish between all five SGARs in terms of their potential for non-targets, and thus they should be treated individually. It is proposed that authorisations are changed such that all can be used in and around buildings; some compounds may be used in open areas under certain circumstances. The proposed change in authorisation requires implementation of a stewardship scheme designed to enhance best practice in terms of use, and thereby reduce non-target exposure. Such changes in authorisation are likely to alter exposure and associated risk to non-targets. This report examines the realistic food intake rates and using derived assessment factors or a combination of all alternatives, when conducting EU risk assessments is appropriate. Pragmatic modifications to risk assessments are already permitted in certain EU member states. It is reasonable to apply similar approaches across the wider EU.

### WE373 Extrapolated LD₅₀ values from limit dose tests for mammals

M. Foudoulakis, Dow Agrosciences / RSRA ERS; E. Bonneris, Bayer CropScience Aktiengesellschaft / BCS AG/EnSa-ETX; L. Lothar Diesing, Bayer CropScience AG; T. Fredricks, Monsanto Company; K. Guth, BASF SE / Ecotoxicology; P.J. Edwards, Central Product Safety Dept; K. Brugger, DuPont Crop Protection

In the EU according to the Guidance Document on risk assessment for birds and mammals (EFSA 2008) where the extrapolation is carried out assuming a 50% binomial probability behind that mortality could be equally divided. This is proposed instead by chance in the test. The outcome of this work contains estimated mammalian LD₅₀ extrapolation factors per number of animals tested at the limit dose.

### WE374 Development of a reproductive risk assessment for birds: LD₅₀/10 vs. dietary NOAEL for evaluation of parental effects

M. Foudoulakis, Dow Agrosciences / RSRA ERS; M. Ebeling, Bayer CropScience AG EnSa-ETX-TV / Environmental Safety Ecotoxicology; D. Sprenger, BASF SE; R. Murfitt, Syngenta Ltd / Environmental Safety; T. Fredricks, Monsanto Company; K. Brugger, DuPont Crop Protection

A new regulatory tool is proposed for plant protection products in the EU according to the EFSA GD (2009), the LD₅₀/10 is used in the reproductive assessment for birds to take account of the possibility of reproductive impairment due to sublethal parental effects e.g. on pair formation or nest-building, not covered in the reproduction study. The objective of this work was to evaluate the use of a short-term dietary NOAEL as refinement for the acute LD₅₀/10 in the reproductive risk assessment for birds. From these dietary toxicity data, one 5-dietary NOAEL (lowest) and one avian repro NOAEL (lowest) were extracted and used in the calculation to the acute oral LD₅₀/10 for the respective compound. Evaluation indicates that the 5-dietary NOAEL may be employed as a surrogate for the LD₅₀/10 and the next lowest endpoint would likely be the avian reproduction study NOAEL. Therefore it is reasonable to apply a similar approach for avian long term risk assessment according to EFSA (2009), where dietary toxicity data are already available.

### WE375 Accumulation and temporal trends of organohalogen contaminants in finless porpoises (Neophocaena asiaeorientalis) collected from Korean coastal waters between 2003 and 2010

E. Jeong, Hanyang University; E. Kim, W. Jeong, A. Shen, Hanyang University; Y. Az. Research Institute (CRI), National Fisheries Research and Development Institute; S. Choi; H. Moon, Hanyang University / Marine Sciences and Convergent Technology

Limited information is currently available on temporal trends of persistent organic pollutants (POPs) in Korean coastal waters. In our study, the concentrations of persistent organic pollutants (PCBs, DDTs, HCHs, and CHLs) and polybrominated diphenyl ethers (PBDEs) in 77 blubbers of finless porpoise (Neophocaena asiaeorientalis) were determined to evaluate the contamination status and temporal trends of POPs between 2003 and 2010. The concentrations of OCs (PCBs, DDTs, HCHs, and CHLs) and PBDEs (in ppb) were determined in blubbers collected in 2010 ranging from 51 to 3200 ng/g lipid weight (lw).
Risk assessment, risk management and mitigation for pesticides: from regulation to public perception (P)

WE376 Minimum detectable differences (MDD’s) in mesocosm studies: The importance of pre-treatment management and appropriate sampling techniques for insectsicides A. Blakey, Cambridge Environmental Assessments; S. Taylor; S. Priestly, Cambridge Environmental Assessments The Aquatic Guidance Document (EFSA, 2013) makes a number of recommendations for aquatic mesocosm studies including the reporting of Minimum Detectable Difference (MDD) values alongside No Observed Effect Concentrations (NOEC). In addition, it is recommended that each study contain at least 8 different sensitive and vulnerable groups for which robust statistical evaluation is possible in order to be able to derive robust the Ecotoxicological Threshold Option (ETO) or the Ecological Recovery Option (ERO) – Regulatory Acceptable Concentrations. Critical to the derivation of robust endpoints is the appropriate establishment of replicate mesocosms to represent diverse and numerous populations and communities known to be sensitive and vulnerable to the effects of the pesticide of interest. Furthermore, in-life sampling techniques must be appropriate for the collection of the exposed organisms at an abundance sufficient to ensure reasonable MDD values can be derived from the data, whilst also ensuring that all ecological niches are sampled. Here we will show the evolution of study design focussing on the pre-treatment phase and the resulting endpoints the to CAMERAS study in Cambridge Environmental Assessments (CEA) whilst drawing on examples from a number of recent mesocosm studies in order to demonstrate how robust data may be better derived from studies with insecticides.

WE377 The Minimum Detectable Difference (2): Appropriate experimental design and endpoint selection for algae in mesocosm studies F. Pickering, Cambridge Environmental Assessments; S. Taylor; A. Arenas-Sanchez, S. Priestly, Cambridge Environmental Assessments Algal communities have a very dynamic nature and are in a constant state of flux with complex interactions which are poorly understood. We are sensitive to a number of different variables which makes it difficult to pinpoint true dose related effects when evaluating responses following exposure to pesticides. Therefore, it is tempting to speculate that the ecological relevance of endpoints for phytoplankton from mesocosm studies based on effects at the species level could be considered to be over-conservative due to the natural variation. In contrast, recent guidance on the conduct and interpretation of replicate mesocosm tests highlights the importance of using the most appropriate aquatic communities for the chemical being investigated. In addition, it is equally the selection of appropriate measurement endpoints and ecological evaluations is critical to the derivation of powerful statistical results with reasonable Minimum Detectable Difference (MDD) values. Here we will show the evolution of study design and endpoint selection for algae in regulatory mesocosm tests using examples drawn from recent state of the art studies with herbicides and cypermethrin. We will provide an analysis of the available data for algae (phytoplankton and periphyton) comparing the respective reliability of the data generated at various taxonomic levels in being able to robustly measure effects and recovery in freshwater mesocosms following exposure of algal communities to pesticides. We will also provide recommendations for further work in order to still improve the robustness of the endpoints for use in regulatory risk assessment.

WE378 Toxicity of the fungicide ketoconazole to freshwater microalgae and cyanobacteria A. Arthenuus, M. Andersson, University of Gothenburg / Department of Biological and Environmental Sciences; L. Eide, WEQA AB; T. Porsborg; T. Backhaus, University of Gothenburg / Department of Biological and Environmental Sciences Antimycotic pharmaceuticals are widely used for treating fungal infections in humans and animals. They enter the aquatic environment either after passage through the body, or after being rinsed off if they are topically applied. However, substantial knowledge gaps currently hamper a proper environmental risk assessment of the individual antimycotics and their mixtures for marine and freshwater environments. Clotrimazole, a marine priority pollutant, affected sterol composition of marine microalgal communities already at 50 pmol/L which indicates effects already at environmental concentrations. In the present study we focus on ketoconazole (also an azole fungicide) and toxicity to freshwater microalgal communities. A concentration response curve was first established using pigment profiles (HPLC) as endpoint which resulted in an EC50 of around 1 micromol/L. During autumn 2014 we repeated the study but focused on the lower concentration range from 0.0001 to 1 micromol/L. Results on chlorophyll a show similar patterns as in the initial study. The experiments were finished during autumn 2014 and are currently under final evaluation and steroid analysis, species determinations and analysis of effects on the lipid composition of algae are currently evaluated. The work is performed within the Swedish Formas-funded project “Aquatic Environmental Risk Assessment of Antimycotics”.

WE379 Species specific differences in sensitivity to prochloraz induced synergy of α-cypermethrin toxicity N. Cederberg, K. Dahlhoff, V. Serrat, University of Copenhagen / Department of Plant and Environmental Sciences Azole fungicides are known to enhance the effect of other xenobiotics, there being the pyrethroid insecticides, Field studies have also shown azole fungicides to be present more or less continuously during the growing season. The concentrations are however, rarely exceeding 10 µg/L and are mostly lower than 1 µg/L. The question is whether these low azole concentrations do also enhance the effect of pyrethroids? And whether there is a large difference between species in their sensitivity to synergistic compounds? In this study we test the effect of a five hour α-cypermethrin pulse at three concentrations over seven days in the continuous presence of the azole prochloraz at 0.1, 10, 50 and 100 µg/L. The setup was carried out using cultures of the three sensitive species and an untreated control. The results showed that six days after a 1µg/L α-cypermethrin exposure, concentrations as low as 1 µg/L prochloraz increased mortality from the app. 15% control mortality to 100% in Daphnia magna. Contrary to D. magna where the prochloraz concentrations alone had no effect on mortality (6-day LC50 for prochloraz was 4476 µg/ prochloraz/L), both H. actae and C. riparius were very sensitive to prochloraz showing 6 day LC50-values of 3.33 and 41.4 µg prochloraz/L. Accounting for the prochloraz toxicity in itself, synergy in relation to independent action predictions, was also observed for H. actae and C. riparius. Though these data are preliminary, they indicate that azole synergy does take place also at quite low and realistic concentrations, and that species specific differences in sensitivities to an azole as prochloraz are quite larger with EC50 values varying more than 1000-fold.

WE380 Future challenges in sediment toxicity testing for the risk assessment of plant protection products D. Eber, B. Buns, Bayer CropScience AG / BCS D ETX Ecotoxicology Sediment toxicity is gaining an increasing awareness within the scientific community. In 2015 a scientific opinion on environmental risk assessment (ERA) for sediment organisms will be published by the European Food Safety Authority (EFSA). This scientific opinion will be of high interest because so far, only tier I RA for sediment organisms is available in the existing aquatic guidance document published in 2013 by the OECD. In order to ensure a well established standardized and validated test guidelines under the umbrella of OECD is limited. Aquatic invertebrates are covered by the OECD technical guidelines (TGs) 218/219/233 for chironomids (Chironomus riparius), representing acute, chronic and full life-cycle tests, respectively, and the OECD TG 225 using the oligochaete Lymnira bavarica in a 21-day chronic test. Aquatic macrophytes are covered by publications of Myriophyllum spicatum (OECD TG 239). In North America the question of sediment toxicity is handled differently as reflected by the ASTM and US EPA guidelines. In general a higher number of test methods with sediment organisms is available (e.g. Hyallela azteca, Chironomus dilatus, Daphnia magna) in the USA and Canada. Furthermore these guidelines are developed under a different scope as compared to the OECD TGs e.g. use of aged spiked sediment under flow-through conditions vs. use of “freshly” spiked sediment in a static system). Another major difference is the use of natural sediment in ASTM and US-EPA guidelines instead of artificial sediment in OECD TGs. The resulting discrepancies between Europe and North America are influencing the potential use of all existing guidelines within one RA approach. The communication therefore will analyze pros and cons of sediment toxicity test design: static vs. flow-through conditions, use of “freshly” spiked sediment (in a static system).
Influence of crop management practices on zonal avian risk assessment of plant protection products. Case of study: a systemic pesticide applied as seed treatment on sugar beet


Regulation 1107/2009 includes the concept of the zonal evaluation, and the subsequent mutual recognition of authorizations in order to guarantee the commercialization of Plant Protection Products (PPP) in the EU and avoid duplication of work. The risk assessment of plant protection products on birds and mammals is determined using the exposure document of European Food Safety Authority (EFSA) on Birds & Mammals (2009). If the uniform principles are not met at the first tier risk assessment steps, a list of refinement options are described in this guidance including i) the identification of focal species, ii) the proportion of diet (PD) of these species taken from treated areas (iii) the residue levels of pesticide in this diet and iv) the time that focal species spend feeding in treated area (PT). As long as these parameters need to be refined, the zonal harmonization between European zones is becoming more difficult because they will depend on specific agro-climatic conditions. This is more challenging for seed treatment applications because its evaluation is considered under an inter-zonal scheme. In this work, we present the main difficulties identified to harmonize zonal evaluations for seed treatments using as example a hypothetical systemic pesticide applied on sugar beet seeds. The results show the time spent on the sugar beet (spring or autumn depending on geographical situation) and the associated crop management practices were critical for establishing the worst-case exposure scenario for avian risk assessment and the ecological parameters (PD and PT) of focal species. In conclusion, together with information on ecological data and residues levels of pesticide (which are currently being investigated by EFSA), it is also necessary to characterize the specific agromonical conditions of each crop and member state level before to set out procedures for zonal evaluation.

WE382


Soil functioning depends on many different microbiological activities and effects. Fungi and bacteria are present in soils and play a crucial role in organic compound and mineralization of organic compounds (including agrochemicals and xenobiotics), but also synthesise organic compounds (e.g. antibiotics, gums), immobilize nutrients, contribute to soil aggregation or serve as nutrient source for the grazing microflora. Furthermore mycorrhical fungal species mediate the transport of water and ions from soil to plants or facilitate plant/plant exchanges of small organic compounds. Soil microorganisms are classified as mycorrhically fungal, mycorrhizal or free-living. The question arises if N-mineralization alone gives an adequate answer if soil functioning is affected by PPP. We have analyzed data from active ingredients with fungidical activity for C- and N-mineralization of the Swiss ecotoxicological database (holding data on formulations and active ingredients). Results are presented and further needs for risk evaluation on microorganisms are discussed with respect to all microbial functions mentioned above.

WE383

Is it safe to recycle home & garden pesticide containers? C. Ramwell; S. Beulke, FERA / Food and Environmental Safety Programme; K. Thompson Food and Environmental Research Agency; T. Allen, FERA, DEFRA/ACRO Ltd; M. Kennedy, E. Bradley, The Food and Environment Research Agency.

The increasing use of ready-to-use (RTUs) pesticide products and the need for recycling to increase means that government advice regarding the disposal of home and garden pesticide containers – rinse and put in the ‘black bin’ - may no longer be appropriate. The aim of our project was to assess the risk to humans and the environment of disposing of used, amateur pesticide containers via recycling, and to compare the risks identified with those arising from landfill and incineration which would result from disposal via a dustbin. The study generated a quantitative model comparing the predicted human or environmental exposure to an exposure threshold to assess the likelihood of an adverse risk occurring. The environmental risk associated with the disposal of residue in part-filled bottles and rinseate from empty bottles was predicted to be below the trigger threshold for four of the five compounds tested. The calculations indicated a moderate risk for fish and algae arising, with and without rinsing, for the remaining compound. Human exposure associated with the disposal of RTU bottles was consistently below the acceptable operator exposure limit (AOEL). Human exposure associated with the disposal of concentrate bottles was consistently below the AOEL for all disposal routes, as long as there was no spillage of partially-filled bottles. UK policy has now been amended to allow the recycling of empty RTU home and garden pesticide containers.

WE384

Pesticides in Swiss surface waters - The gap between risk assessment and monitoring

B. Fulda, Agroscope; A. Aldrich, Research Station Agroscope ACW / Ecotoxicology; R. Kasteel, M. Balmer, T. Poiger, Agroscope

The transport of pesticides from agricultural areas to adjacent non-target environments, in particular surface water systems, is estimated for the ecotoxicological risk assessment prior to the registration of pesticides. In Switzerland the pesticide concentrations in surface waters are predicted by environmental realistic worst-case models, considering pesticide leaching via drainage, surface runoff, and spray drift. The entry via drainage and runoff is simulated with EXPOSIT 3.01 (German Federal Environment Agency, UBA) whereas drift is calculated using the basic drift values after Rautmann et al. (1999). These models are designed to be ‘conservative’, representing the edge of the field and should therefore result in higher predicted pesticide concentrations (PEC) than would actually occur in the environment. A comparison between the PEC of a pesticide and the measured environmental concentrations (MEC) from field monitoring studies can be useful to ascertain potential model deficiencies and provide a valuable feedback loop to the registration process. In this study we compared a large set of Swiss surface water monitoring data from 2005 – 2012, consisting of 29 sampling campaigns at 107 monitoring sites. The results show that in many cases PEC exceedances were found, concerning only few substances and sampling locations. However, the ratios between PEC and MEC were highly dependent on various critical factors as for example the assumed entry pathway, the selection of the appropriate PEC-scenario from the multiple authorized indications or, in case of mitigation options like no-spray buffers zones. In addition, the validity of results is also restricted by limitations in the monitoring data for example partially low sample frequency, inadequate sampling approaches to capture peak concentrations and a high fraction of non-detects that may also be associated with lack of application in the catchment area or in the relevant time period. The results of such comparisons of exposure model vs. monitoring data must therefore be interpreted with caution.

WE385

The Swiss re-evaluation process of authorised plant protection products: Outcome of the ecotoxicological risk assessment

A. Aldrich, Research Station Agroscope ACW / Ecotoxicology; R. Gauch; E. Kohlschmid, Agroscope / Institute for Plant Production Sciences IPS; K. Lautenschlager, D. Ruf, Agroscope Changins-Wädenswil; M. Plath, O. Daniel, Agroscope / Institute for Plant Production Sciences IPS; S. Knaure, The ecotoxicological evaluation procedure of plant protection products (PPP) in Switzerland is analogous to the EU. In total about 2000 products and 400 active substances have been authorized in Swiss. To such number of substances are authorized needed about 1.5 years in case of re-evaluation. In 2010 a re-evaluation of a PPP with PEC exceedance resulting from the Swiss regulatory risk assessment. In a first assessment, only few cases of PEC exceedance were found, concerning only few substances and sampling locations. However, the ratios between PEC and MEC were highly dependent on various critical factors as for example the assumed entry pathway, the selection of the appropriate PEC-scenario from the multiple authorized indications or, in case of mitigation options like no-spray buffers zones. In addition, the validity of results is also restricted by limitations in the monitoring data for example partially low sample frequency, inadequate sampling approaches to capture peak concentrations and a high fraction of non-detects that may also be associated with lack of application in the catchment area or in the relevant time period. The results of such comparisons of exposure model vs. monitoring data must therefore be interpreted with caution.

WE394

Ecosystem Services Analysis of 1,3-D Used in Integrated Pest Management Strategies in Tomato Production in Italy

N. Furr; S. Deacon, ENVIRON UK Ltd; A. Alish, Dow AgroSciences / Risk Management; E. Tescari, Dow AgroSciences; A. Bartram, AstraZeneca UK Ltd; P. Burston, ENVIRON; M. Rockel, ENVIRON International Corp.; J. Nicolette, ENVIRON International Corporation

This is a case study addressing the use of the soil fumigant 1,3-dichloropropene (1,3-D) within integrated pest management strategies (IPM) for the cultivation of fresh tomatoes in southern Italy. Food production is both economically and culturally important to European farmers, particularly in Italy where farmers grow flavonomeous tomatoes with passion and pride. Growers rely on pesticides for crop
Amounts of exposure in gloves and hands were 0.0037% in and as of arms, hand and hip (total amount of front and back) and legs were 0.0021%, 0.00266% and 0.00213%, respectively. Inhalation exposures were less than 0.00001%. Total exposures including all the body parts and inhalation were less than 0.011%. Risk assessment was carried out by calculating MOS. The MOS in this experiment was more than 1, indicating a low possibility of risk.

WE390 Pesticide residues related risk on human and environment in Turkey E. CAMCI, Adnan Menderes University / Faculty of Agriculture Lab Environmental Toxicology, M. Yalçın, Adnan Menderes University / Faculty of Agriculture; C. Turgut, ADU; A. Gavcar, Adnan Menderes University / Faculty of Agriculture Lab Environmental Toxicology, S. Mermer, Fac. of Agriculture Lab

EXO

Various diseases in humans are occurred by the increased industrialization, people life style who migrated to the city with the progress of science and technology, being away from nature, formation of exclusive natural products, working life which topics should be taken into account, what kind of consumption habits do people have and so on should be investigated.

WE391 Alterations on synapsin immunostaining in mushroom bodies of africanized honeybees Apis mellifera (Hymenoptera, Apidae) exposed to sublethal dose of Thiamethoxam. T. Roat, Biology; A. Catac, Sao Paulo State University /UNESP / Biology; P. Frade, Sao Paulo State University /UNESP; P. Cunha, Sao Paulo State University /UNESP / Biology; R. Nocelli, Federal University of Sao Carlos /UFSCar Araras; P. Nunes, Sao Paulo State University /UNESP; O. Malapinsa, Universidade Estadual Paulista

Honeybees (Apis mellifera L., 1758) are important pollinating insects giving to this species a great agronomic, environmental and economic relevance. However the recent development in the number and diversity of particularly bee species, may threat food security and ecosystem integrity. Many stress factors acting alone or together, can weaken the colony including the decreased availability of resources due to the degradation and fragmentation of honeybee habitat, pathogens and parasites, beekeeping management, and the use of chemical pesticides on crop. Thiamethoxam is a neurotoxic insecticide, acting as an agonist of acetylcholine and is extensively used in sugarcane cultivation which are large crops a fazenda Rio Bonito State. Therefore, this study aimed to investigate how the brain of these insects reacts to the intoxication by sublethal dose LD$_{50}$ of thiamethoxam. For this, we analyzed possible changes in synaptic pattern in the mushroom bodiess of africanized honeybees by immunostaining of the protein synapsin (1 SYNORF ANT1) conjugated to CY5 (secondary antibody) and it was used DAPI for nuclei staining. Using the software LAS-AF with the Confocal Laser Scanning Microscope Leica TCS SP5-II, it was possible to measure the intensity of emitted fluorescence in the areas of the mushroom bodies. The synapsin labeling was more evident in the mushroom bodies of bees exposed to the sublethal dose (0.0027 ng of thiamethoxam/bee) for until 8 days was incubated with three monoclonal antibody against the protein synapsin (1 SYNORF ANT1) conjugated to CY5 (secondary antibody) and it was used DAPI for nuclei staining. Using the software LAS-AF with the Confocal Laser Scanning Microscope Leica TCS SP5-II, it was possible to measure the intensity of emitted fluorescence in the areas of the mushroom bodies. The synapsin labeling was more evident in the mushroom bodies of bees exposed to the sublethal dose specifically in the regions of glia, compared with the control group. Thus, the sublethal dose of the insecticide thiamethoxam intensified synapsin immunostaining, suggesting an increased release of neurotransmitters, which may be linked to neurotoxicity, and overexcitation. These results provided information about which brain structures are affected by thiamethoxam, which suggest possible physiological and behavioral consequences that such exposure may cause in the insect that can affect the intra-colonial relations. Financial Support: Fapesp (2012/13370-8)

WE392 Monitoring of pesticides in groundwater - First steps of a SETAC group to develop a guidance A. Gimsg, The Danish Environmental Protection Agency / Pesticides and Gentechnology; W. Tütting, German Federal Office of Consumer Protection and Food Safety (BVL); A. Bozin, ANSES

The SETAC Environmental Monitoring Advisory Group of Pesticides (EMAG-Pest) deals with post-registration studies and monitoring data for an evaluation of environmental effects of Plant Protection Products. It is structured into four working groups: Terrestrial vertebrates, terrestrial invertebrates including pollinators, aquatic invertebrates and fish, terrestrial plants and soil and groundwater. The aim of the groundwater (GW) working group is to develop a guidance on groundwater monitoring for regulatory purposes. The experts’ first outcome on the topic was to develop a guidance which topics should be taken into account, what kind of consumption habits do people have and so on should be investigated.
Monitoring data in regulatory context: Monitoring data can be used in the regulation of pesticides, but use of such data is not straightforward. Key question: When should a groundwater study be triggered/recommended and what questions should a monitoring study answer? Monitoring and modelling: When monitoring studies are available, they are always compared with modelling results (FOCUS or local modelling). Key question: Could a groundwater monitoring be used as the highest tier study and supersede a modelling approach? Limitations and strengths of both studies. Groundwater Monitoring “good practices”: The design of a monitoring study and the quality of the data are key to generate valuable monitoring results. Key question: How should a monitoring study be designed so that data can be used for regulatory purposes (demonstrating relevance/representativeness of data, comparison with protection goals)? Interpretation of GW monitoring data / Relevance of sites and results: How long do the tested substances in different sources of the surface water, which were taken from pond, river and/or lake. As a reference item, radiolabelled benzoic acid was used. The presented results cover a comparison of the degradation rates and of the recovery of this reference item within the thirty tests. In addition to these, the hydrolysis, the degradation and the mineralization of the different test items were also compared. Water monitoring at different time points during the field study did not occur often during the experimental studies. Additionally, the effect of suspended solids on the degradation rate was and is still unclear. One important part of the testing, the identification of metabolites, appeared to be very challenging and in some cases more time consuming than expected. In summary, the comparison of the OECD 309 studies, with the different substances with the different characteristics, show that this “standard” test can be simple but can very complex as well. From a C&O perspective, this leads to different workloads and therefore different prices of the same type of study, which has to be reflected in the study price.

WE396 Use of public large scale monitoring data for pesticide risk assessment: issues, needs and propositions
A. Duboisset, A. Boivin, E. FARAMA, ANSES; V. Poulsen, ANSES / French Agency for Food Environmental and Occupational Health
Safety Water monitoring can be used as the highest tier information for pesticide risk assessment prior to registration (Regulation EC 1007/2009; FOCUS 2009). Large scale public monitoring, routinely undertaken by water agency and/or government bodies, provide consistent criteria may be required to ensure consistency of water data assessment and related decision making processes. Yet differences noticed between the conclusions drawn from public monitoring datasets and regulatory assessments give rise to debates about pesticide occurrence in water. This presentation explores why datasets from public monitoring are not easy to handle by regulatory authorities for drawing up risk assessment conclusions. It aims at briefly setting out the background that underlies such issue: e.g. divergence between the objectives and rationales of the various stakeholders, and between the different regulations involved; the way how existing large scale water monitoring databases are built and updated at FR level. A short summary of the basic data processing that might be needed to allow such databases to comply with the main objectives of regulatory risk assessment is then provided. Since no guideline on this issue is available at EU level, it emphasizes the need to draw up common EU guidance to assist regulatory authorities and applicants on the way to handle public monitoring data within regulatory procedures for risk assessment. In that view, the place of public routine monitoring (within tier scheme) and their use would need to be discussed. To provide a support for such discussion, two options are considered as example: public monitoring data and SOPs, or public databases and SOPs. The first option (i) can be provided as background information to the risk manager (e.g. current practice in Anses opinions for the national renewal of PPP in France) or (ii) may trigger additional information in case of positive detections to identify their cause (e.g. German national guidance).Whatever the use of such public monitoring databases, specific approach including reliable statistical methods (e.g. Leterme 2006) and the definition of consistent criteria may be required to ensure consistency of data interpretation. In this regard, proposals from an approach recently developed in France (EIS project: Gauroy et al., 2012) are given.

WE397 Prospective environmental risk assessments - are we on the right track? H. Sandberg, M. Klar, J. Axelmand, Swedish Chemicals Agency
In order to place a plant protection product (PPP) on the market it has to be demonstrated that the PPP doesn’t cause unacceptable effects to non-target organisms. This is demonstrated by prospective risk assessment methods, which are performed by the applicants. The methods have been developed and harmonized within EU. The methods should be valid – they should measure what they claim they measure. And, they should be reliable and ensuring equal treatment for all applicants. The competent authority of the member state where the PPP is to be placed on the market evaluates these risk assessments and makes decisions based on the evaluations. The focus of this analysis is on the validity and reliability of the method. This analysis does not address the degree of protectiveness of the method. Considering the uncertainties associated with monitoring data, it is important to foresee adverse effects in the environment as well as the low reliability (different conclusions from different stakeholders), the appropriateness of the method within a regulatory context is hampered. Standardising the process, reducing negative impact of expert judgment and using fewer but robust predictors can enhance reliability. Nevertheless, the environmental complexity and uncertainties we are unable to consider can still result in an overall low value validity, even for an improved prospective risk assessment method. Consequently, there is a need for caution when making assumptions about general applicability of data from specific conditions. It is crucial that we understand and acknowledge the limitations with prospective risk assessments, that we adapt our methods accordingly and that the limitations are transparently communicated.

WE398 Ten years after the Stockholm Convention: What do atmospheric monitoring data tell about its effectiveness in reducing organochlorine pesticides?

W. Völkel, Innovative Environmental Services Ltd; S. Hogen, Innovative Environmental Services (IES) Ltd / Environmental Toxicology
The chemical fate of substances and also the fate of their metabolites is becoming an important part of the environmental risk assessment. One of the required and standardized tests is the aerobic mineralization of substances in surface water. The testing procedure is described in the OECD testing guideline 309, which has now existed for 10 years in its current version. Since every substance is different to each other, this guideline can only be a framework for the real testing procedure. During the last three years we have performed 30 tests following the OECD testing guideline 309. All test items were labelled and up to three labels per substance have been tested. The water solubility of the substances varied between the low µg/l and the g/l range, and they were dissolved in water or organic solvents. Volatile substances were also amongst the tested substances. We tested the behaviour of the substances in different sources of the surface water, which were taken from pond, river and/or lake. As a reference item, radiolabelled benzoic acid was used. The presented results cover a comparison of the degradation rates and of the recovery of this reference item within the thirty tests. In addition to these, the hydrolysis, the degradation and the mineralization of the different test items were also compared. Water monitoring at different time points during the field study did not occur often during the experimental studies. Additionally, the effect of suspended solids on the degradation rate was and is still unclear. One important part of the testing, the identification of metabolites, appeared to be very challenging and in some cases more time consuming than expected. In summary, the comparison of the OECD 309 studies, with the different substances with the different characteristics, show that this “standard” test can be simple but can very complex as well. From a C&O perspective, this leads to different workloads and therefore different prices of the same type of study, which has to be reflected in the study price.
H. Wöhrnschimmel, ETH Zurich; M. Scheringer, ETH Zuerich; C. Bogdal, ETH Zurich; K. Hungerbuehler, ETH Zurich / Institute for Chemical and Bioengineering In 2014, ten years have passed since the UNEP Stockholm Convention on Persistent Organic Pollutants (POPs) entered into force. The objective of this Convention is to protect human health and the environment from the detrimental effects of POPs by reducing their emissions, where a majority of the substances originally listed for elimination are pesticides. According to the convention, the effectiveness of the reduction measures is to be evaluated by monitoring POPs in different environmental media. For ambient air, monitoring programs such as EMEP and the UNEP Global Monitoring Plan have collected comprehensive data sets of atmospheric concentrations. Here, we present a time series analysis on organochlorine pesticide concentrations in ambient air. We systematically search for trends, the source of persistent structures and trends that might result from the provisions of the Stockholm Convention. We find that structural changes do occur in time series of atmospheric concentrations, but they seem to be related not only to effects of the Stockholm Convention, but also to national regulations enforced prior to the implementation of the Stockholm Convention. Also effects of chemical transport and fate, as for instance the revolatilization of POPs from tropospheric clouds, may be the reason for some of the observed structural changes. We conclude that ten years of data are not yet sufficient for detecting any general and statistically significant effects of the Stockholm Convention. Based on these lessons we derive recommendations for the future operation of global monitoring programs, and advocate for a stricter implementation of the precautionary principle, in the current absence of proof for the effectiveness of the Stockholm Convention.

WE399 Post-harvest uses of Plant Protection Products: case of study for development of a harmonized environmental risk assessment for non-agricultural uses A. Haro, INIA National Institute for Agricultural and Food Research and Technology / Technical Directorate for Evaluation of Plant Varieties and Plant Protection Products; P.V. Gonzalez, Centro de Investigaciones del Medio Ambiente, Dto. Quimica, Facultad de Ciencias Exactas, UNLP, CONICET; C. Jorquera, INIA National Institute for Agricultural and Food Research and Technology / Technical Directorate for Evaluation of Plant Varieties and Plant Protection Products; E. Prades, INIA; J.L. Alonso-Prados, INIA. National Institute for Agricultural and Food Research and Technology / Technical Directorate for Evaluation of Plant Varieties and Plant Protection Products The zonal evaluation of non-agricultural uses of plant protection products (PPPs) under the framework of Regulation 1107/2009 is not harmonised. The first step in the environmental risk assessment is to consider the likelihood of exposure. Currently, there is a lack of understanding of defining emission pathway diagrams in relation to the type of application in order to rapidly detect the concerned environmental compartments. In this work we present a methodology to cope with this type of uses considering post-harvest treatment as a case of study. Under Regulation 1107/2009, post-harvest treatment is considered as interzonal evaluation and currently there is no harmonised risk assessment scheme at zonal level for this kind of use. Post-harvest processes are integrated in the food production system involving an outstanding economic and health valueler. These processes include the use of plant protection products (PPP) to prevent pests and diseases during the storage and commercialization of commodities. PPP intended to be used in post-harvest treatments have been considered as indoor treatment with a negligible environmental exposure. However, post-harvest technology is an industrial process with a high consumption of water which implies waste water emission that may lead to exposure to different environmental receptors. In this work we propose an emission scenario to quantify the exposure to the different concerned environmental compartments for post-harvest uses based on the work and knowledge from risk assessment approaches developed for biocides and industrial chemicals, as an attempt for harmonization of environmental risk assessment approaches at EU level for this kind of uses.

WE400 EFSAs opinion on good modelling practice and stakeholders’ perceptions of ecological models: expectations vs. reality M. Plath, L. de Baan, O. Daniel, Agroscope / Institute for Plant Production Sciences The spread of pesticide resistant fungi, weeds and insects is the result of synergistic effects between natural selection and the selective pressure of pesticides. Pesticides primarily kill sensitive target organisms while promoting the survival and reproduction of the less susceptible ones. Thus, the usage of pesticides, particularly the application of the same active ingredient of or active ingredients with the same mode of action over the long-term, may foster the resistance of the target organisms. Information from Plant Variety Approval Procedures may therefore provide an important measure to assess the pesticide resistance risk in individual crops. In Switzerland, an agri-environmental monitoring system was launched in 2009. Within this monitoring, pesticide usage surveys are conducted annually. About 300 farmers voluntarily report their pesticide usage, including applied product, amount, time and crop for each pesticide application. We hypothesized that these usage data may be used to deduce the risk of potential pesticide resistance in specific crops by evaluating the diversity of active ingredients and related modes of action used. A set of diversity indices were tested for their suitability to serve as pesticide resistance risk indicator. Low diversity was assumed to be indicative for a high pesticide resistance risk. The applied active ingredients were grouped according to their mode of action, as defined by the Resistance Action Committee for fungicides (FRAC), insecticides (IRAC) and herbicides (HRAC). First results revealed significant differences across crops regarding the diversity of pesticide used, suggesting that certain agricultural systems may undergo an increased risk of pest resistance. A yearly update of such an indicator could provide information on the long-term development of potential risks of pesticide resistance and could be assessed alongside with the long-term development of the ecotoxicological risk of the applied active ingredients. However, further tests are required to validate the use of diversity indices as possible pesticide resistance risk indication.

WE401 Why time matters: A stock-pollution approach to regulating PBT/vPvB chemicals S. Gabbert, Wageningen University / Social Sciences; L. Hilber, Agroscope ART A core aim of the chemicals’ legislation in Europe is that hazardous chemicals are adequately controlled. For chemicals for which an adequate control of the risks is considered not possible decision-making on the use or non-use is increasingly based on a socio-economic analysis (SEA). A SEA is expected to allow for a more balanced decision, where the gains from keeping a chemical in use are opposed to expected damage costs for society. Here we argue that for chemicals which are, in addition to their toxicity, persistent and/or bioaccumulative (PBT/vPvB chemicals), the weighing of benefits against costs must account for stock pollution effects, causing environmental concentrations in a compartment to increase over time. We demonstrate how stock pollution effects can be incorporated into a SEA. For this purpose we first develop a dynamic model for assessing stock effects in an environmental compartment. The model illustrates that stock dynamics, i.e. the increase of environmental concentrations over time, depend on the interplay of the initial concentration, the release rate, and a chemicals’ degradation half-life. Including the model into a cost-benefit analysis approach allows identifying the regulatory decision for the use or non-use of PBT/vPvB chemicals. For this purpose we first develop a dynamic model for assessing stock effects in an environmental compartment. The model illustrates that stock dynamics, i.e. the increase of environmental concentrations over time, depend on the interplay of the initial concentration, the release rate, and a chemicals’ degradation half-life. Including the model into a cost-benefit analysis approach allows identifying the regulatory decision for the use or non-use of PBT/vPvB chemicals. For this purpose we first develop a dynamic model for assessing stock effects in an environmental compartment. The model illustrates that stock dynamics, i.e. the increase of environmental concentrations over time, depend on the interplay of the initial concentration, the release rate, and a chemicals’ degradation half-life. Including the model into a cost-benefit analysis approach allows identifying the regulatory decision for the use or non-use of PBT/vPvB chemicals. For this purpose we first develop a dynamic model for assessing stock effects in an environmental compartment. The model illustrates that stock dynamics, i.e. the increase of environmental concentrations over time, depend on the interplay of the initial concentration, the release rate, and a chemicals’ degradation half-life.
WE403 Aquatic Risk Assessment for Triazole Fungicides: The Impact of Epoxiconazole and Tebuconazole on Aquatic Fungi L. Wiesner, Goethe University Frankfurt / Department Aquatic Ecotoxicology; L. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy and Cell Biology; M. Junghans, Swiss Centre for Applied Ecotoxicology EAWAG - EPF / Ecotox Centre

The risk of fungicides to aquatic fungi has been disregarded in aquatic risk assessment so far. Besides classically tested aquatic organisms like fish, crustaceans, algae and higher plants, aquatic fungi like hyphomycetes or yeasts play an important role in the food web dynamics of aquatic ecosystems. Therefore, this study aimed at investigating the release of fungicide residues from standard aquatic fungi and yeast using a standard sorption kinetic method to assess the possible influence of fungicides like epoxiconazole and tebuconazole on aquatic fungi. Two test setups were used: (i) a growth test with the aquatic yeast Cryptococcus laurentii (Basidiomycota) and (ii) a growth test with the hyphomycete Tetrachaietum elegans (Ascomycota). In the 96-well plate bioassay with C. laurentii an EC50 value of 0.0458 mg/L and an EC10 value of 0.0073 mg/L was derived for epoxiconazole. For tebuconazole the EC50 and EC10 were 0.131 mg/L and 0.126 mg/L respectively. This is in accordance with literature data for the aquatic yeast Cryptococcus flavescent. A species sensitivity distribution (SSD) with acute toxicity data from aquatic vertebrates, invertebrates, algae and higher plants shows C. laurentii, besides Lemna gibba (0.144 mg/L) as the most sensitive organism for tebuconazole. For epoxiconazole, the growth test with the aquatic hyphomycete T. elegans resulted in an EC50 of 0.0329 mg/L, which is similar to the yeast EC50 of 0.0458 mg/L. Due to time constraints the yeast test could only be carried out twice, therefore the results need to be verified, and additional influencing factors should be investigated; e.g. smaller mycelia starting size led to an increase of test sensitivity. Since cellulose filters were used as organic substrate a decrease in filter-colonisation was observed in the presence of epoxiconazole. Both tested fungal species are among the most sensitive aquatic organisms for triazole fungicides. Hence, the consideration of aquatic fungi in triazole fungicide risk assessments is of great importance. With regard to the growth endpoint, the yeast bioassay is the more promising test, because it has a shorter test duration and allows for the testing of a higher number of replicates in the same run. Since sporulation might be a more sensitive parameter than growth, a hyphomycete test system under continuous bioassay needs to be developed for assessing the chronic toxicity of fungicides on aquatic fungi – unfortunately we were not able to induce sporulation in the hyphomycete strain. Reduction strategies of micropollutants from large and small-scale sewage water facilities (P)

WE404 Natural zeolite as an alternative sorbent for the toxic dye Rhodamine 6G removal Z.I. Yaneva, Trakia University, Faculty of Veterinary Medicine / Chemistry; N. Georgieva, Trakia University / Chemistry

Introduction Rhodamine 6G (R6G, Basic red 1) is a cationic fluorescent azo dye applied widely in textile industry, as a genetic marker/cell staining in biochemistry. Its carcinogenicity, reproductive and developmental toxicity, neurotoxicity and chronic toxicity to humans and animals was experimentally proven. Thus, the removal of R6G from industrial wastewaters is important in terms of protection of public health, environment and aquatic life. The aim of the present study was to present an alternative methodology for the removal of R6G from aqueous solutions by using natural zeolite. Materials R6G (C28H34N2O12Cl) was obtained from Sigma-Aldrich. Dye concentrations were determined spectrophotometrically at 524 nm. The standard curve was linear over the range of the tested concentrations (R² 0.9996). The natural zeolite used in the present study was supplied from deposits in Bulgaria. Zeolites surface chemistry and morphology were characterized by Boehm titration, pH point of zero charge (pHpzc), FTIR and digital microscopy analyses. Results The sorption characteristics of R6G on the natural sorbent were investigated in a batch mode in the concentration range 5.0-35.0 mg/L, with sorbent mass 0.5 g and equilibrium time 24 h. The UV/VIS spectra of R6G within the pH range 2-5 did not display shifting of the absorption peaks. The intensity of the absorption maximum at pH 5.8 was the highest. The pHpzc of natural zeolite was 7.35. The digital microscope images of the fresh and dye-loaded zeolite displayed samples heterogeneity and irregular distribution of dye macromolecules on the solid particles surface. The positions of the peaks in the FTIR spectra of the fresh and R6G-loaded zeolites were approximately similar but the bands intensities of the dye decreased with the increase in the mass ratio of dye/zeolite towards R6G was 2.8 mg/g. Experimental data showed a good fit with the multilayer model (R² 0.9953). It allowed the calculation of both the monolayer sorption capacity (1.625 mg/g) and the equilibrium constants for the first layer (Kₛ 49.218) and subsequent (Kₛ 0.0688) adsorption. The monolayer capacity of zeolite according to the Langmuir model was 2.79 mg/g. The highest established extend of the dye uptake Kₛ 49.218% with removal efficiency 93% was achieved after 1 h of contact time and an availability, all the monolayer capacity of dye uptake capacity natural zeolite can be successfully applied for R6G removal from aqueous solutions. Keywords: Rhodamine 6G, natural zeolite, sorption

WE405 Isoniazid removal from water by adsorption on acid-modified zeolite N. Georgieva, Trakia University / Chemistry; Z.L. Yaneva, Trakia University, Faculty of Veterinary Medicine / Chemistry; S. Stanilova, D. Petrova, Trakia University; M. Staleva, University Prof. Assen Zlatarov

Introduction Isoniazid (INH, Rimiteron), a first-line medication used in the treatment of infection caused by Mycobacterium tuberculosis, is commonly found in the group of "emerging" pollutants. Released into the environment, these pharmaceuticals biologically active substances are active even in small amounts as they pollute natural waters and destroy or inhibit the activity of certain microorganisms important for the ecosystems. The aim of the present study was to investigate the physiochemical, morphological characteristics and adsorption capacity of acid-modified zeolite as cost-effective alternative adsorbent for isoniazid removal from water. Methods INH concentrations were determined by an adapted UV/VIS method at 262 nm. The standard curve was linear over the range of the tested concentrations (R² 0.9996). The natural zeolite used in the present study was supplied from deposits in Bulgaria. It was modified with 4M HCl. Zeolite surface chemistry and morphology were characterized by determination of pH of zero charge, FTIR and digital microscopy analyses. Results The analysis of the experimental results established that the highest extend of INH uptake during the kinetics study was E 95.5 %, achieved at initial drug concentration 100 mg/L, mass of zeolite 1 g and contact time 320 min at 22°C. The pseudo-second order kinetic model displayed an extremely high degree of correlation (R² 0.9996). Thus, the rate-limiting step during INH adsorption on zeolite might be chemisorption involving bond formation between the drug molecule and zeolite. The highest equilibrium zeolite capacity towards INH was 7.17 mg/g. The Sips model best represented the experimental equilibrium data (R² 0.980) of the drug sorption on zeolite. The integrative analysis of surface chemistry/sorption studies showed that the probable sorbate-sorborbent interactions include van der Waals forces, H-bonds and chemical interactions between INH functional groups and zeolite mineral groups, and that there is a parallel intraparticle diffusion of drug molecules in the mesopores of the mineral particles. In conclusion the unique properties of zeolite and the established high extend of INH sorption proved the possibility of its successful application for the removal of the drug from aqueous phase. Keywords: isoniazid, emerging pollutants, zeolite, sorption Acknowledgements: The study was supported financially by Project NoSF-40/UP, Trakia University, Stara Zagora, Bulgaria.

WE406 Pilot scale ozonation of problematic wastewater contaminants: clarithromycin, 1H-benzotriazole and carbamazepine - Consequences regarding ecotoxicity and transformation products M. Posselt, Department of Applied Environmental Science ITM; M. Radke, Institute for Hygiene and Environment / Environmental Laboratory; M. Breitzhoft, Inst für tillämpad miljövetenskap / Department of Applied Environmental Science ITM; P. Schwartz, Federal Environment Agency Germany; T. Meinelt, Leibniz Institute for Freshwater Ecology and Inland Fisheries

Oxidation has been proposed as a technology for quaternary wastewater treatment to improve the removal of anthropogenic trace contaminants. However, wastewater ozonation under economically feasible conditions does not necessarily result in complete oxidation of organic compounds but can instead lead to the formation of largely unknown transformation products (TPs). Results from several studies suggest that certain TPs might have a higher ecotoxicological potential than the original substances. Therefore, the goal of this study was to investigate the consequences for aquatic ecosystems. What is missing in particular are data from combined approaches comprising both advanced analytical as well as ecotoxicological test methods. In this study we investigated the effects of the TP mixtures from three organic micropollutants (clarithromycin (CL), 1H-benzotriazole (BZ), carbamazepine (CBZ)) on different test organisms according to OECD TGs: 201 (D. subspicatus), 221 (L. minor) and 236 (D. rerio). In parallel, we searched for TPs in ozonated samples using HPLC/Q-TOF. Removal efficiencies and test concentrations were measured by UHPLC MS/MS. O₃ treatment was carried out at a pilot plant integrated into a municipal sewage treatment plant (STP) at Stockholm, Sweden. Either effluent water (EW) from the STP or tap water (TW) and ozone treated water were ozonated, aerated and finally sand filtered. We applied retention times (20 min) and O₃ doses (0.6 g O₃/g TOC) that were previously described as economically feasible for large-scale application in STPs. Ozonation of EW controls and TW controls revealed no adverse effects in ecotoxicity tests. CL was removed to >99% from both EW and TW. CBZ was completely removed from EW. BZ revealed as more resistant treatment with removal efficiency 67% and TP mixtures of BZ and CL were either less toxic than the parent substances or non-toxic in ecotoxicity tests. Sandfiltration did not entirely remove TPs and the overall removal of parent compounds was not significantly improved. CL-N-oxide was identified as the main TP of CL. In contrast to the parent substance, its toxicity towards algae was negligible. Our data suggest that EW ozonation is an effective tool to address the problem of critical CL concentrations in surface waters reaching the tentative recommended environmental quality standard (QSₚₑ,ₑ,max) of 0.130 mg/L as well as to improve the removal of CBZ and, to a minor degree, BZ.

WE407
Benefit of wastewater treatment plant upgrading with activated-carbon filters for connected freshwater ecosystems

P. Thellmann, H. Köhler, Tübingen University / Animal Physiological Ecology; K. Wurm, GOL Water Ecology Laboratory Starzach; R. Triebsock, University of Tübingen / Animal Physiological Ecology

Advantages of wastewater treatment plant (WTP) upgrading with additional cleaning stages as, e.g., ozonation or powdered activated-carbon (PAC) filtration, are often a breaking topic in sanitation, environmental engineering, and the efficiency of the available technologies for the reduction of micropollutants and pathogens is a matter of intense scientific discussion. Whether or not this reduction of chemicals does result in an improvement of ecosystem health, however, is far from being understood. The two WWTs Ebingen (located at the Schmiecha River) and Lautlingen (located at the Eyach River), both in Southern Germany, have been upgraded with powdered activated-carbon filters more than 20 years ago, at that time aiming at reducing stains released by local textile industry from the effluents. Prior to the upgrade, the rivers Schmiecha and Eyach were highly polluted (“stained in all colours”) and biota were hard to find. After the upgrade of the two WWTPs, the quality of the receiving water courses has continuously improved. Nowadays, pollution by stains has decreased dramatically due to close-down of textile industry. As a result, the reduction of “modern” micropollutants by active-carbon filtration has more and more moved into the focus of interest. In order to show the long-term efficiency of the WTP upgrading for the connected rivers Schmiecha and Eyach in this context, we investigated ecosystem health upstream and downstream of the two WWTs by means of biomarker analyses in fish (including investigations of tissue signatures, stress protein levels, and micronuclei in blood cells) for developmental toxicity, assessment of the fish population structure, and by calculation of ecological indices. The results indicate a healthy status of fish and invertebrates both upstream and downstream of the two WWTs, and reflect the high efficiency of the powdered active carbon stages for sewage effluent cleaning and, thus, for ecosystem integrity of the rivers Schmiecha and Eyach.

WE408 Thermally Activated Persulfate Removal of Cyclohexanecarboxylic Acids and Radical Mechanisms

X. Xu, Universidad Autónoma de Madrid; J.E. Silva, Ingeniería Química; G. Pliego, J. Zoio, J. Casas, Universidad Autónoma de Madrid; J. Rodriguez, Autonomous University of Madrid

Nowadays, increasing attention has been paid on the environmental impact of Nap hentic acids (NAs) from oil sands process-affected water (OSPW). NAs are series of alicyclic carboxylic acids with extreme toxicity. In the current work, cyclohexanecarboxylic acid was chosen as the model pollutant to be treated by thermally ac activated persulfate oxidation. A reaction kinetics model was constructed based on the radical mechanisms involved. An initial CHA concentration of 50 mg/L was used. The amount of persulfate was varied from 0 to 100% of the stoichiometric dose toward CHA. The effect of working temperature was considered. Radical mechanisms were investigated by adding scavengers (tert-butyil and et hanol) to the systems, and the effect of chloride (14.3 mM) ions were investigated. The autoxidation of CHA at pH 8 and 100 °C was 2.8 mM perulide under 80 °C. The rate of TOC removal was well described by a simple pseudo-first order rate equation. Sulfate and hydroxyl radicals were believe to be the reactive species for the degradation of NAs, and the former were verifi ed to be the dominant radicals. The evolution of hydroxyl radicals was studied by adding quenching agent at different reaction times. Chloride was proved to be of little impact toward the removal of CHA.

WE409 Ecotoxicological assessment of domestic wastewater treated by membrane technology

P. Palma, Instituto Politécnico de Beja / Department of Technologies and Applied Sciences; S. Figado, Politécnico Institute of Beja; C. Santos, CEBAL; G. Palma, C. Cavaco, R. Gomes, EMAS; L. Neves, CEBAL

With the increment of world’s population and the consequent demand for water supply, the sustainable development and the conservation of water resource will require the reuse, recycling and the improved quality of wastewater discharges. In these systems, the removal processes, may be considered an efficient and economic technology, which could be integrated in wastewater treatment plants (WWTs) for the increment of the physical, chemical and ecotoxicological proprieties of the wastewat. The aim of the present study was to characterize and evaluate the efficiency of nanofiltration (NF90, NF270) and reverse osmosis (SW30) membranes, in improving the quality of the effluent from the WWT Mine of Louro (Portugal), both in Southern Portugal. The results indicate that the three membranes have high removal percentages for the most parameters studied. All parameters concentrations are below the legal limits, according to the Decre Decree N.º 152/97 of 19 June, except the total nitrogen, with the NF270 membrane. With respect to the ecotoxicological effects, in general, the use of membranes removed the toxicity detected in the initial samples (classified as Class 2 of UT). The SW30 membrane showed a greater toxicological efficiency removal comparing with the nanofiltration membranes. Further, among the nanofiltration membranes used, the NF90 promoted a greater decrease in the toxicity of the samples. Thus, treatment with the membranes NP90 and SW30 allowed obtaining an effluent with high quality and low ecotoxicological potential, without endangering the environmental balance in the water resource.

WE410 Assessment of the hydraulic performance of activated sludge plants and its influence over estrogen degradation

T. Coello Garcia, T. P. Curtis, Newcastle University / Environmental Engineering; P. H. Butterworth, Newcastle University / CEGS; W. Mrozek, Newcastle University / School of Civil Engineering Geoscience; R. Davenport, Newcastle University / School of Civil Engineering and Geosciences

Natural and synthetic estrogens have been detected worldwide in wastewater treatment plants effluents. They have been reported to be responsible for endocrine disruption and, although currently unregulated, they are on an EU monitoring list for prospective legislation, with a proposed environmental quality standard (EQS) for E2 and EE2 of 0.4 and 0.035 ng/L respectively. This increases pressure on the water industry to implement highly expensive tertiary treatment technologies in their wastewater treatment plants (WWTP) to comply with these standards. There is evidence that activated sludge (AS) processes can achieve the requisite removal of estrogens, but its optimisation is poorly understood. Operational parameters such as the solids retention time (SRT) and the hydraulic retention time (HRT) have been suggested to influence estrogen degradation, but this is difficult to assess on full-scale plants due to the variations in flow and insufficient control. Furthermore, the hydraulic performance of the aeration tank (i.e. completely stirred tank reactor (CSTR) or plug flow) has not always been taken into account. A series of tracer studies were performed in several aeration tanks in conventional AS WWTPs to gain information on their real HRT and hydraulic regime. The results of these studies were fitted to the dispersion and tanks in series models, and the Aquasim software was used to account for recirculation in the systems. Typical influent concentrations and degradation constants from the literature were used to predict the removal of estrogens in these reactors based on the observed HRT and hydraulic regime. Thus, for influent concentrations between 2.4 and 150 ng/L for E2 and 0.5 and 70 ng/L for EE2, and degradation constants between 0.174 and 5.75 h^-1 for E2 and 0.006 and 0.1 h^-1 for EE2, in the worst case scenario the difference in the percentage of removal among a CSTR and a plug-flow accounts for as much as a 16%, which, at low influent concentrations, was enough to ensure an effluent concentration below the EQS. The results also showed that the observed HRTs were considerably shorter than the hydraulic retention time (HRT) in the tank, which appreciably varies the predictions for removal rates, thus emphasizing the importance of its accurate determination. This assessment points out that some existing WWTPs would not necessarily need major investments in tertiary treatment, but an improvement in their hydraulic performance, to meet the required standards.

WE411 Non-target analysis and prioritization of compounds emitted from on-site sewage treatment facilities

K.M. Blum, Umea University / Department of Chemistry; P.L. Anderegg, Umea University / Chemistry; B. Björnles, G. Renman, KTH Royal Institute of Technology; P. Haghund, Department of Chemistry

Many households in Sweden are not connected to any central sewage treatment plant and therefore use on-site sewage treatment facilities (OSSFs). The emissions of micropollutants to surface and ground waters from these facilities are largely unknown, which calls for a thorough effluent and removal efficiency characterization. Influent and effluent samples were collected from Swedish on-site sewage treatment facilities (OSSFs), including: soil beds, miniaturized sewage treatment plants (STPs) and source-separated systems. In addition a set of samples were taken from medium sized municipal STPs (without industrial input) and one large municipal STP. OSSFs influents or effluents from facilities with similar treatment technique were pooled after filtration. Pooled filter and water samples were analyzed using Solid Phase Extraction with toluene extraction and dichloromethane extraction with dichloromethane, respectively. The corresponding water and filter extracts were combined and analyzed with comprehensive gas chromatography time-of-flight mass spectrometry. Full-scan spectra were compared to the NIST mass spectral library for preliminary identification. Manual investigation of peaks with >65 % spectra similarity assured the spectral quality of the library. Matrix effects were excluded if they had elevated blank levels, originated from column or septa bleed or had a low detection frequency. Environmental fate parameters for the tentatively identified compounds were estimated with the EPISUITE in silico tool by the US Environmental Protection Agency and use information was extracted from EU and national databases. Priority ranking according to persistence, bioaccumulation, toxicity, concentration levels and removal efficiencies resulted in a final list of environmentally relevant chemicals discharged from OSSFs. Amongst the priority compounds were food additives, fragrances, detergent ingredients, cosmetic additives, plastic additives and biocides. These will be targeted in a recently started project entitled RedMic aiming at improving the OSSF treatment technologies and reducing diffuse emissions from OSSFs.
WE412 Active biomonitoring to evaluate efficiency and potential impacts on coastal area from submarine discharge point of an industrial integrated waste water treatment plant
f. Perin, Thetsis Ltd; L. Corsi, University of Siena / Physical Earth and Environmental Sciences; E. Primel, Universidade Federal do Rio Grande; K. Boltes, University of Alcala / Chemical Engineering; A. Pun, University of Alcala / Chemical Engineering; P. LETON, Univ. ALCALA; A. Esteve, University of Alcala / Chemical Engineering; J. Salas, J.R. Pidre, Fundación Centro de Nuevas Tecnologías del Agua (CENTA); L. Nozal, IMDEA Water
In 2014, in the framework of an integrated monitoring program, mussels (Mytilus galloprovincialis) originating from the same population grown in a mussel farm, were transplanted for 30 days near and far from the treated water submarine input and at a reference site. Summer and Winter exposure experiments were performed and samples analyzed for organic micro-pollutant and metal contents and biomarkers in different organs. Bioaccumulation of IPA, PCB, chlorinated pesticides and metals were carried out in whole soft tissue of mussels. As for biomarkers, stress on stress and Condition Index were determined as general index of animal status. Catalase, Malondialdehyde and Total Metallothionein content were measured in digestive glands while lysosomal membrane Stability (NNRT) and micronecrosis in haemocytes. The chemical and biological results will be elaborated in order to provide an evaluation of health condition of animals related to the contamination level and indirectly of the surrounding environment. The data, responding to the legislation requests related to EIA, will contribute to define management strategies to be used to evaluate the treatment efficiency and ecofeasibility of similar industrial integrated waste water treatment plants.

WE413 Optimization of parameters of small wastewater treatment facilities, the OP-TREAT project
H. Ejhed, IVL Swedish Environmental Research Institute; J. Maeng, IVL Swedish Environmental Research Institute Ltd; E. Dorgelo, PIA RWTH Aachen University; G. Plaza, Institute for Ecology of Industrial areas
Onsite wastewater treatment is of great concern due to the load of phosphorous and nitrogen contributing to the eutrophication of the recipients. The load of household related hazardous substances as pharmaceuticals, personal care products and antibiotic resistant genes are of further concern, since even very low concentrations can cause effects on the ecosystems and contaminate drinking water resources. The WHO has recently identified the increased spread of antibiotic resistant genes as one of the largest threats to mankind in the future. However, onsite wastewater treatment optimized to mitigate wastewater pollution, could offer cost-efficient solutions. In remote areas and areas lacking infrastructure, onsite wastewater treatment is the only alternative. This work presents results from a sand-filter bed spiked with a pharmaceutical cocktail of fifteen substances, showing more than 60% reduction of most substances, but 30% reduction of the calciturbance carbamazepine. Thus, the results implicate that onsite wastewater treatment could have better or equally good reduction efficiencies as many conventional active sludge WWTP. However variations in the reduction efficiencies in this study as well as in literature reveal the need for in-depth studies and optimization of the parameters driving the reduction processes. This BONUS financed project continues to focus on the physico-chemical and technical aspects of optimizing the processes driving the onsite wastewater treatment systems. Wastewater treatment is performed by combinations of mechanical, chemical and biological treatment. Simulated concentration tests on daily flow pattern, and investigating all those factors from bacteria so on an optimized system were formed using three type techniques allowed for testing at the European test center for certified small wastewater of treatment facilities in Aachen, Germany. The tests aim at a holistic assessment of reduction efficiencies of: nutrients, pathogens, pharmaceuticals, personal care products and antibiotic resistant genes. Parameters that are monitored and varied are primarily: pH, redox, temperature and residence time. Results will lead to a benchmark state of the art of the parameters controlling the reduction efficiencies in the onsite wastewater facilities, allowing for optimization to meet the demands of reducing risk of contaminating recipients and drinking water.

WE414 Strategies to Reduce Diffuse Emissions of Micropollutants from On-Site Sewage Treatment Facilities
P.L. Andersson, Umea University / Chemistry Department; E. Kärman, KTH Royal Institute of Technology / Land and Water Resources Engineering
Nearly 700 000 private households in Sweden are not connected to any central sewage treatment facilities and thus organic micropollutants (MPs) from on-site sewage treatment facilities (OSSFs) are in large unknown. The recently initiated research RedMic is devoted to develop strategies and techniques to reduce emissions of MPs from OSSFs. The overall aims are to 1) identify and quantify emissions of MPs from OSSFs, 2) develop and evaluate novel innovative OSSFs techniques, 3) identify major sources of MPs emitted from private households, and 4) suggest an efficient strategy for reduction of diffuse emissions from private households. In this presentation the initial findings will be presented on the development of a multi-criteria analysis as a basis for a decision support strategy for management of diffuse emissions from OSSFs. A step-wise multi-criteria analysis approach will be undertaken including 1) definition of system boundaries, 2) selection of criteria covering ecological, economical and social dimensions, 3) selection of system alternatives i.e. possible options for reducing MPs including source control, changes in human behaviour and technical improvements, 4) prioritisation and weighting of the criteria in a workshop with a stakeholder group, and 5) comparison of the different options using the criteria from step 2. The final model comparison will be done using substance flow-, cost- and energy analysis and also qualitative assessment. Hazardous substances will be discussed including aspects as biodegradation, soil beds and small scale sewage water treatment techniques with various additional reduction steps including ozone treatment and charcoal filtration, applicability, life cycle costs, operation, maintenance, emission reductions, and regulatory aspects.

WE415 Development of Microbial Electrogenic Technologies for removal of emerging pollutants from wastewater
K. Boltes, University of Alcala / Chemical Engineering; A. Pun, University of Alcala / Chemical Engineering; P. LETON, Univ. ALCALA; A. Esteve, University of Alcala / Chemical Engineering; J. Salas, J.R. Pidre, Fundación Centro de Nuevas Tecnologías del Agua (CENTA); L. Nozal, IMDEA Water
Emerging pollutant are not completely removed in current sewage treatment systems so they become recalcitrant. The problem is even more relevant if we take into account that an important amount of wastewater associated to small and rural populations (< 2 000 eq-hab) is not treated at all. This population (around 3-4 million inhabitants inSpain) still do not have access to any kind of wastewater treatment so emerging pollutants as well as pathogenic are directly released into the environment. That small populations show a range of technical and economic features that makes low-energy and simple operation treatments the most appropriate for treating their wastewater. Wastewater technology based on biological treatments requires a suitable electronic acceptor for consuming the electrons generated in the microbial metabolism of organic matter. Microbial electrogenesis consists in using of conductive material for the cathodic and the anodic reaction of microorganisms expanding their substrate of substrates. In this work, we evaluate the role of microbial electrogenesis in the biodegradation of emerging pollutants. Our strategy is not just limited to carry out assay at lab scale. Since 2010, the authors have been working together for coupling microbial electrogenesis with extensive technologies as artificial wetlands. Our research team has developed a full scale artificial electric wetland which is dairy fed with urban wastewater and placed inside the PROTONA facilities (Carrión de los Céspedes, Seville). We have evaluated the removal capability of our system in field and at lab scale, measuring a set of 15 emerging pollutants (Atenolol, Paraxanthine, 4-AAA, 4-DAA, Codeine, Caffeine, 4-AAA, 4-FAA, Sulfamethoxazole, Citalopram HBr, Carbamazepine, Ketoprofen and Naproxen) by OC-MS analysis coupled by a set of toxicity test using different aquatic organisms (algae, plants). Our results show that electric wetlands are a very interesting tool to treat wastewater effectively and sensitive material on emerging pollutants biodegradation under real conditions.

Commuter air quality in rail submarine systems: current understanding and future mitigation (P)

WE416 First appraisal of atmospheric organic contaminants in Brazil using XAD-2 resin-based passive samplers
P.G. Costa, FURG / Escola de quimica e alimentos; K.S. Miglioranza, University of Mar Del Plata / Lab of Ecotoxicology and Environmental Pollution; F. Wania, University of Toronto / Physical Earth and Environmental Sciences; E. Primel, Universidade Federal do Rio Grande; G. Fillmann, Universidade Federal do Rio Grande / Institute of Oceanography
Patricia Gomes Costa1,2, Maiara Dutra, Thaila Wille1, Sergiane Alves2, Karina S. Miglioranza1, Frank Wania1, Ednei Gilberto Primel3, Gilberto Fillmann3
1Laboratório de Microcontaminantes Orgânicos e Ecotoxicologia Aquática, www.conco.furg.br 2Laboratório de Atmosfera e Cidades, Suelos e Microorganismos, patcosta0@gmail.com 3Laboratório de Microcontaminantes Orgânicos e Ecotoxicologia Aquática, www.conco.furg.br Keywords: passive air sampling, POPs, XAD-2, Brazil
We have been working together for coupling microbial electrogenesis with extensive technologies as artificial wetlands. Our research team has developed a full scale artificial electric wetland which is dairy fed with urban wastewater and placed inside the PROTONA facilities (Carrión de los Céspedes, Seville). We have evaluated the removal capability of our system in field and at lab scale, measuring a set of 15 emerging pollutants (Atenolol, Paraxanthine, 4-AAA, 4-DAA, Codeine, Caffeine, 4-AAA, 4-FAA, Sulfamethoxazole, Citalopram HBr, Carbamazepine, Ketoprofen and Naproxen) by OC-MS analysis coupled by a set of toxicity test using different aquatic organisms (algae, plants). Our results show that electric wetlands are a very interesting tool to treat wastewater effectively and sensitive material on emerging pollutants biodegradation under real conditions.
Global Monitoring Plan (GMP) for POPs. Of the pesticides measured, concentrations in air were dominated by PCBs, DDTs, endosulfan (and its metabolites in low concentrations) and the current-use pesticides (CUPs): Dichlofluanid, chlorothalonil, atrazine and trifluuralin. Other pesticides that were regularly detected included α- and γ-hexachlorocyclohexanes (HCHs) and dieldrin. PAHs, PBDEs and emerging contaminants (bisphenol-A, iprodione, ipfolg, tertbutyl-cyclohexylparaben) were present in many samples. These analyses are consistent with previous studies in the same region. Some Ammonia contributed to the increase significant the spatial and temporal resolution in a sustainable manner to cope with long-term studies. Some of Brazil results (mainly background sites) were incorporated in the second Global Monitoring Report, which will be presented for the 2014 Conference of the Parties of the Stockholm Convention on Persistent Organic Pollutants.

WE417
Air quality in stations and trains in the Barcelona subway system V.I. Martins, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); T. Moreno, Geosciences; M. Minguillón, X. Querol, Institute of Environmental Assessment and Water Research (IDAEA-CSIC). People living in metropolitan areas usually spend a considerable amount of their daily time commuting. In today’s society, the subway is a well-developed and highly promoted transportation mode because of its comfort, high speed, environmental friendliness, and large transport capacity. However, prior studies in subway systems of several cities worldwide indicated that particulate matter (PM) concentrations are generally lower on the platforms, in two different seasonal periods (warmer and colder), attributed to the air ventilation settings. The PM produced in the tunnels is frequently transported into the city efficiently and without contaminating the air. However, the question arises: we know that city outdoor air is contaminated, but what about the indoor air of the subway and trains? Could these measured in ambient air. The passengers are exposed to indoor air potentially polluted by various sources, including the abrasion of rail tracks, wheels, catenary and brake pads produced by the motion of the trains, and the movement of passengers which promotes the mixing and suspension of PM. An extensive measurement campaign was performed in the Barcelona subway system in order to characterise PM monitoring its contribution into investigating trains and on platforms, in two different seasonal periods (warmer and colder), to better understand the main factors controlling it, and therefore the way to improve air quality. The subway system comprises eight subway lines, at different depths, with different tunnel dimensions, stations designs and train frequencies. Four subway stations were selected for daily measurements and supplementary samplings were also performed in a total of 20 additional stations. Chemical composition of a total of 236 daily PM2.5 filters sampled in the 4 different platforms was also investigated. Measurements inside the trains were carried out in six subway lines. PM concentrations varied widely among underground stations, according to different ventilation and air conditioning systems, characteristics of each station and variation in the traffic and the number of passengers entering into the subway system. The platforms were generally lower than those in the conventional system, due to more advanced ventilation set up, but also to lower train frequency and the higher ventilation rates in the trains. PM concentrations on trains were also performed in a total of 20 additional stations. Chemical composition of a total of 236 daily PM2.5 filters sampled in the 4 different platforms was also investigated. Measurements inside the trains were carried out in six subway lines. PM concentrations varied widely among underground stations, according to different ventilation and air conditioning systems, characteristics of each station and variation in the traffic and the number of passengers entering into the subway system. The platforms were generally lower than those in the conventional system, due to more advanced ventilation set up, but also to lower train frequency and the higher ventilation rates in the trains. PM concentrations on trains were generally lower than those on the platforms, attributed to the air conditioning systems operating inside the trains, which are equipped with air filters.

WE418
Implementing Methodologies and Practices to Reduce air pollution Of the subway environment - IMPROVE LIFE T. Moreno, Geosciences; E. de Miguel, TMB; X. Querol, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); M. Capdevila, S. Centelles, TMB; V.I. Martins, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); M. Minguillón, C. Reche, IDAEA CSIC Barcelona. The greatest 21st century challenge to air quality in our cities is the pollution produced by road traffic. In contrast, underground rail systems provide an environmental urban lifeline, enabling large numbers of people to commute through the city efficiently and without contaminating the air in the streets above. However, the question arises: we know that city outdoor air is contaminated, but how clean is the air breathed underground? How much does the traffic pollution reduce down the newly built tunnel sizes? The answer is: how much the movement of the trains themselves contribute to the inhalable particulate loading in the tunnels and on platforms? The question of air quality in underground rail transport is not trivial: hundreds of millions of commuters in cities across the world use such systems daily, so any improvement in platform and train air is likely to produce significant health benefits. In this context, the European Union has recently awarded the IMPROVE LIFE project (Implementing Methodologies and Practices to Reduce air pollution Of the subway environment) LIFE project to the Air Quality Research Group at IDAEA-CSIC, and the local transport authority Transports Metropolitanos de Barcelona (TMB) with the clearly defined objective of providing local and national transport authorities of European countries with a protocol for recommended measures to enable them to reduce concentrations of particulate matter and improve air quality in metro platforms and trains. The results obtained so far indicate that air quality in stations is highly variable, depending on the complex interplay of such factors as station design and ventilating systems, and even the chemistry of brake pads used by the trains. Train frequency, number of people on the platform, depth of the station and its access to traffic-contaminated streets above all additionally affect air quality. IMPROVE LIFE will produce an air mitigation strategy document appropriate for underground transport systems, and distribute it to transport authorities across the world. Apart from the obvious relevance to Europe, where around 60 cities have underground rail systems, the CSIC group is particularly interested in communicating their findings to countries in east Asia, notably Japan, South Korea, and China which is rapidly building new metro systems. Keywords: metabolic and physical properties of the subway - subway PM, platform and train air quality. Acknowledgements: IMPROVE LIFE project (LIFE13 ENV/ES/000263) is funded by the European Community.

WE419
Air quality in old and new subway systems of Barcelona from analysis of organic molecular compounds B.L. van Drooge, J.O. Grimalt, Environmental Chemistry; V.I. Martins, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); T. Moreno, Geosciences; M.C. Minguillon, CSIC; X. Querol, Institute of Environmental Assessment and Water Research IDAEA-CSIC; L. Navarrormatin, CSIC; B. Pina, IDAEA-CSIC / Environmental Chemistry This work presents the analysis of organic molecular tracer compounds in PM2.5 samples collected at 4 subway platforms of the Metropolitan area of Barcelona during 2013 and 2014. Whereas the platforms of Joanic, Santa Coloma and Tetuan stations belong to the old conventional system, Llefia is part of the new system. The subway in Llefia is a single tunnel with one rail track separated from the platform by a wall with mechanical doors (PSDs) that are opened simultaneously with the train doors. It includes advanced platform ventilation systems and has drivers train with optimised speed, braking and stopping controls. PM2.5 samples were collected on quartz microfiber filters by means of a high volume sampler that was situated on the platforms. The filters samples were analysed following a described procedure. 40 organic molecular tracer compounds were quantified in the PM2.5 samples, including toxic compounds, such as PAH, but also source-specific compounds, such as those from traffic, fragrances, tobacco smoke, wood, burning, plastics and dust. Multi-variance analysis of the data base resolved five emission sources. The organic aerosol (OA) at the conventional stations were dominated by primary combustion sources in summer (45-80% of OA), while the OA in the new station was dominated by plasticizers (70% of OA) probably from the building material. Samples collected in winter showed contribution of outdoor biomass burning in all stations from 50% of OA in the stations further away from the city center, while the contribution was <10% in the city center. In this period also the contribution of indoor dust mixed with fragrances was a dominant role in OA contributions in two conventional stations (30% of OA). The results showed large season and spatial variability among the stations, although generally, the stations of the new subway showed lowest levels of organic compounds, as well as lowest responses in complementary biological tests (fish embroy toxicity). These tests showed relatively mild toxic effects, and biological responses only related to combustion sources and dioin-like compounds, which had higher contributions in the older subway systems.

WE420
ASSESSMENT OF RESPIRABLE PARTICULATE MATTER IN ISTANBUL SUBWAY SYSTEM U. Alver Sahin, Environmental Engineering Department; B. Onat, Istanbul University / Environmental Engineering Department. Previously conducted studies investigating the relationship between particulate matter and daily mortality and morbidity in passengers were exposed to this risk in the short and the long term due to the endangerment of their health. People spend most of their time indoors; either at home, or in the workplace. One of the places with elevated risk of dust exposure is the transportation system. The underground transportation system is one of the convenient modes of transportation in many metropolitan cities. In this study, PM10 concentrations, as well as the size distributions of Fe-containing and Cu-containing particles were measured for weekly periods of 7 days throughout 24-h in 6 subway stations of the underground transportation system in Istanbul. The average daily PM10 concentrations were determined as 85.7 μg/m³, 136 μg/m³ and 240.9 μg/m³ at three stations in M1 line: 4. Levent, Sisli and Taksim, respectively and as 67.8 μg/m³, 58.6 μg/m³ and 112.8 μg/m³ at the stations of the new underground metro system, which includes Aksaray and Bakırköy stations. All concentrations were significantly higher (p<0.01) than those that were monitored in the urban air stations. The average Fe and Cu concentrations in total PM were 10.3-28.2 μg/m³ and 134.7-325.4 μg/m³, respectively at the stations of M1 line. The relative abundance of Fe-containing particles among the particles of size ≥2.1 μm collected in line M1 metro stations was determined as 15-30%, which is 3.5-8 times higher than the abundance determined in the Istanbul atmosphere.

WE421
Effect of depth in the Air Quality of a Mexican Subway V. Magica, Universidad Autonoma Metropolitana / Basic Sciences; J.M. Murcia, O. Hernandez, A.A. Neria, Universidad Autonoma Metropolitana - Azcapotzalco / Tacubaya subway station, located close to downtown of Mexico City, was selected to conduct a study to determine the presence and metal content of fine particles at different seasons and platforms’ depths. In this station, three lines have correspondence: Line 9 at 10 m depth, Line 1 at 25 m depth and Line 7 at 40 m
depth. Monitoring campaigns using High Vol samplers were carried out in dry-cold and dry-warm seasons. PM₂.₅ concentrations in line 9 were 67 and 68 µg/m³ during dry-cold season and warm-dry season respectively; in line 1 were 99 and 108 µg/m³ respectively, and finally 107 and 126 µg/m³ station in line 7. The PM₂.₅ inside the platfrompresentedconcentrations between 9 and 100% higher than outside. Statistical analysis showed significant difference among Line 9, and the other two Lines (1 and 9) with different depths, but there was not significant differences between cold-dry and warm-dry season, although in general higher concentrations of PM₂.₅ were measured in the warm-dry season. The analysis of particle showed high concentrations of iron, copper, chromium, and zinc which can contribute to oxidative stress in cells.

**LCA of nanotechnology-based products (P)**

**WE424**

Multi-Criteria Decision Analysis assessment of Sustainable Nanotechnologies A. Zabeo, v. subramanian, Ca Foscari University of Venice / Dept of Environmental Sciences Informatics and Statistics; E. Senzenig, Ca Foscari University of Venice / Environmental Sciences Informatics and Statistics; D. Hristovsz, Ca Foscari University of Venice; I. Linkov, US Army Engineer Research and Development Center; A. Marcomini, University of Venice / Department of Environmental Sciences Informatics and Statistics

The adoption of Engineered Nanomaterials (ENM) in the production of everyday goods is constantly increasing all over the world generating therefore a growing attention on its sustainable management and use. Sustainability requires the assessment of many different aspects related to the production, use and disposal of Engineered Nanomaterials like ecological and human health risks, environmental impacts, as well as costs and benefits of nano-products. Sustainability can be improved by selecting the most effective risk management alternatives (e.g. safety by design technological alternatives, personal protective equipment) taking into consideration the most relevant multiple criteria and indicators of evaluation. The presented Multi-Criteria Decision Analysis assessment methodology is aimed at aiding decision makers in the selection of effective management measures and technological alternatives for risk reduction by properly integrating results coming from the application of the most up to date methodologies related to the evaluation of ecological and human health risks (risk analysis, RA), environmental impacts (Life Cycle Assessment, LCA), costs as well as benefits of nano-products (Cost Benefit Analysis CBA) in the light of decision maker’s preferences. The presented methodology has been developed in the frame of the European Commission’s FP7 framework project SUN (http://www.sun-sfp7.eu/) that aims at developing a comprehensive framework, assessment methodology and Decision Support System (DSS) software for the evaluation of sustainable nanotechnologies.

**Managing nanomaterials in the environment: lessons learnt to date, state of the art and future perspectives (P)**

**WE426**

Integrated discriminant biomarker analysis as a tool to reveal the effects of metallic nanoparticles on the freshwater mussel Dreissena polymorpha following mesocosm exposures

M. Garaud, Laboratory LIEC - CNRS UMR 7360 / Udl. / CNRS UMR, S. Devin, LIEC - Université de Lorraine - CNRS / LIEC CNRS UMR, V. Felten, LIEC, M. Auffan, cÉaint / International Consortium for the Environmental Implications of Nanotechnology, C. Bertrand, Université de Lorraine, CNRS UMR 7360; C. Pagnout, Université de Lorraine; S. Pain-Devin, Université de Lorraine - UL / LIEC CNRS UMR; F. Rodius, Université de Lorraine; B. SOHM, P. Rousselle, LIEC - Université de Lorraine - CNRS; M. Tell, CEREGE; P. Wagner, LIEC CNRS Université de Lorraine; L. Gianberini, Université de Lorraine, CNRS UMR 7360 / LIEC CNRS UMR

Metallic nanoparticles (NP) are among the most widely used NP, notably silver NP (nAg) for their antibacterial properties, and ceria NP (nCeO₂) for their catalytic and anti-UV properties. As environmental exposure is increasing, the importance of assessing nanoparticle ecotoxicity in environmentally relevant conditions has been stressed, but the ecotoxicity testing often focused on acute toxicity assessment using standardized protocols and species. Yet, multimarker approaches have been shown to be useful for detecting early impacts of stressors on organisms. However, the analysis and synthesis of the complex biomarker dataset can be difficult and need powerful integrated biomarker analysis tools. In our works, we assessed the sub-lethal effects of nAg and of nCeO₂ on the freshwater mussel Dreissena polymorpha in two distinct experiments. Experiments were conducted in small (5L) mesocosms simulating simplified lotic ecosystems with the presence of a natural bacterial community and of three species of green algae. Tanks were chronically contaminated with the NP during 4 weeks to reach nominal concentrations of 1 mg/L of nCeO₂ and 50 µg/L of nAg at the end of the experiment. The fate of and effects of NP on mussels were measured using a multibiomarker approach. We then performed a linear discriminant analysis on the biomarker data in order to assess the main influencing factors of the main environmental conditions led to different patterns of biological responses and (ii) to select the minimal battery of biomarkers that allow identification of the exposure condition. In both nCeO₂ and nAg experiments, discriminant analysis allowed us to clearly separate experimental conditions. The biomarker datasets were reduced from 24 to 9 for nCeO₂ and to 10 for nAg exposures. Using two discriminant functions with these reduced sets of biomarkers, we were able to identify a key period of exposure in both mussels. Moreover, on the four most discriminative biomarkers (p < 0.05) of each experiments, three were common between nCeO₂ and nAg exposures, namely gill MT and GSTp1 mRNA expressions, and haemocyte lysosomal morphology. It suggests that these biomarkers could be of primary interest in future nanoparticle ecotoxicity assessment. As a whole, the impacted biomarkers were more indicative of an exposure to NP, while toxic effects biomarkers were largely unaffected, suggesting that these metallic NP would not exert significant toxicity on mussels exposed in environmentally realistic conditions.
Ecotoxicology in tropical and polar regions (P)

TH001

Obtaining an Overview of the Environmental and Human Risk Potential of Pesticides in a global perspective based on currently available Data - a Literature Review Study

F. Weiss, Eawag - Swiss Federal Institute of Aquatic Science and Technology / Department of Environmental Chemistry; C. Zurbrügg, Eawag Swiss Federal Institute of Aquatic Science and Technology / Department of Water and Sanitation in Developing Countries; R.I. Eggen, Eawag / Department of Environmental Toxicology; C. Stamm, Department of Environmental Chemistry

Today, pesticides are intensively used in agriculture across the globe. Worldwide, about 2.4 x 10^9 tons of pesticides are used annually on 1.6 x 10^9 ha of arable land. This yields a global average use of pesticides of 1.53 kg/ha year. In future, the use in the agricultural sector will continue to grow. Recently, it was estimated that within the last decade, the world pesticide market increased by 93% and the Brazilian market alone by 100%. Though pesticides are intensively used, scientifically sound data on the effects of pesticide use are still lacking or not available. Furthermore, current agricultural practices often do not consider sound approaches of pesticide application. Therefore it is highly relevant to: i) identify risk areas where pesticides affect environmental and human health; ii) understand the environmental behavior of pesticides in vulnerable ecosystems; and iii) develop possible mitigation options to reduce risk exposure to ecosystems and humans. Information was collected from scientific literature, reports and webpages of specialized organizations and NGOs to obtain a comprehensive overview on the environmental burden of pesticides. Available data show that the highest annual application rates of pesticides, range from 3 to 76 kg/ha arable land, are found in El Salvador, Bolivia, Malaysia, Guatemala, Ecuador, Jordan, Panama, Belize, China, Suriname, North Korea, Colombia, Costa Rica, Mauritania and the Bahamas. Tropical areas thus seem to be important “hot spots” of pesticide use. Nevertheless, especially in low and middle income countries (LAMICs), the available data on pesticide use are not comprehensive enough to obtain an overall picture of the associated risks at global scale. However, literature evidence from single studies show high impacts on environmental and human health. Internationally banned pesticides are still often used or stocked under unsafe conditions in LAMICs. Furthermore, low skills and knowledge of workers on sound pesticide use, storage and handling is widespread. From the human risk perspective it is estimated that around 3 million people suffer from serious pesticide poisoning worldwide. A project has recently been initiated with a focus on assessing pesticide exposure of the aquatic environment and humans in tropical countries of LAMICs. Other aims of this follow-up project are to assess hazards of pesticide exposure and to suggest mitigation options to reduce exposure to pesticides.

TH002

Agricultural impact on water quality and ecosystems of the lower Tempisque River basin, Costa Rica

E. de la Cruz, Universidad Nacional / IRET; M. Pinnock Branford, Universidad Nacional / Instituto Regional de Estudios en Sustancias Toxicas; S. Echeverria, Instituto Regional de Estudios en Sustancias Toxicas; F. Mena, Universidad Nacional / Instituto Regional de Estudios en Sustancias Toxicas; C. Barata, CSIC / Environmental Chemistry; C. Ruespert, Universidad Nacional / IRET.

This project assesses the effects of contaminants from agriculture (rice, sugar cane and pasture) on the habitat, structure and function of wetland communities in the lower Tempisque River Basin, located in the Pacific of Costa Rica. Between 2009 and 2012 a program that monitored the presence of pesticides and nutrients in surface water at nine sites in the area, which were classified from severely to lightly affected, was done. Four of these sampling points (2 were reference and 2 impacted) also have information on acute and chronic toxicity to aquatic organisms, on biomarkers (GST, LPO y AChE in muscle and brain) in field exposed fish (Poecilia gillii); of Daphnia magna feed rate changes after 24 hrs field exposure; of the macrobenthic and plant community structure changes and over 30 physicochemical parameters, including pH, conductivity, Dissolved oxygen, nutrients and grain size sediment. Residues of ametryn, terbutryn, butachlor, dimethoate, diuron, epoxiconazole, propiconazole, tebuconazole, triazophos, cypermethrin, endosulfan-b and hexazinone pesticides water were determined in surface water. At sites where the water enters to the area (little or less contaminated) the contamination was lower or nonexistent and at the areas influenced by crop production contamination by pesticides and nutrients were increased. Changes were observed in the abundance of macroinvertebrates responses and communities’ indices; some associated with pollution. In macroinvertebrates, the disappearance of families such as Perlidae and Platystomatidae, which are at little affected site; and an increase in the abundance of mollusks and oligochates and the appearance of families Caenidae, Glossosomatidae, in severely affected one. Something similar happened with the agal community. Macrophyte species richness and amphibious plants were higher at sites with high content of organic matter and backwaters. The estimated risk for aquatic organisms of some pesticide residues concentrations in surface waters reveals a problem. Most of them were over the security indicator to protect diversity and ecosystem processes.

TH004

Glyphosate and AMPA mixture in a tropical and polar region: An ecotoxicological risk assessment

R. Bonanje, Universidad Nacional de Cordoba / Facultad de Ciencias Quimicas; L. Fialho, Universidade Nacional de Cordoba; D. Marino, Universidad Nacional de La Plata; D.A. Wunderlin, Universidad Nacional de Cordoba / ICYTA DPTO QUIMICA ORGANICA FACULTAD DE CIENCIAS QUIMICAS; M.V. Ame, Universidad Nacional de Cordoba / CONICET / Biorquimica Clinica

Glyphosate is the most widely used herbicide worldwide, in order to evaluate the occurrence of this pesticide and its microbiological degradation product, the ammoniummonophosphoric acid (AMPA), this study measured their presences in freshwater, sediments and suspended particulate matter (SPM) samples from Suquia River basin in Cordoba, Argentina. From all the analyzed samples 35 % contained glyphosate, AMPA or both compounds. The concentrations were spread in different percentages depending on the environmental matrices considered, ranging from 12 to 20 times higher of glyphosate and AMPA in sediment and SPM than in water. The maximum concentrations found in water were 125 µg. l^-1 of glyphosate and 4.8 µg. l^-1 of AMPA, in sediment 1882.3 µg. kg^-1 and AMPA 266.1 µg. kg^-1 and in SPM 1570.7 µg. kg^-1 of glyphosate and 684.9 µg. kg^-1 of AMPA. The most polluted area came to be CM that is situated in a green belt zone, in second place are RP and SR, extensive agricultural areas. As regards the aquatic risk assessment in water and sediments, fish in LC and CM are threatened by the concentrations of glyphosate in water; these values were below the levels of concern for wildlife however it is convenient to update the limits established for freshwater aquatic protection.

TH005

Biomarkers in fish populations in a tropical and polar region

D.A. Wunderlin, Universidad Nacional de Cordoba; E. de la Cruz, Universidad Nacional / IRET; M. Pinnock Branford, Universidad Nacional / Instituto Regional de Estudios en Sustancias Toxicas; S. Echeverria, Instituto Regional de Estudios en Sustancias Toxicas; F. Mena, Universidad Nacional / Instituto Regional de Estudios en Sustancias Toxicas; C. Barata, CSIC / Environmental Chemistry; C. Zurbrügg, Department of Environmental Chemistry; C. Stamm, Department of Environmental Chemistry

The Northern Parana region stands out in the Brazilian agricultural scenario, with a large area of agricultural lands in its entire path and has almost no riparian forest to function as a buffer. This stream has significantly higher nitrate (6.8-17 mg/l) and conductivity (59-70 µS/cm) than all the other rivers in the watershed. Additionally, pesticides have been detected since 2001 in Peje Stream in very similar concentrations than the ones found in the present study (~6 µg/l of bromacil and ~0.05 µg/l of diazinon). This indicates that MI inhabiting Peje Stream must have some degree of tolerance to pesticides. It is noteworthy that some of the families composing the community year round (Hydropsychidae, Baetidae, Elmidae) are not typically classified as tolerant and, also, there is a marked seasonal variation in the abundance of other intolerant families such as Leptohyphidae, Leptohyphidae (Ephebomoptera), Philopomatidae, Glossosomatidae, Hydroptilidae (Trichoptera), Corydalidae (Megaloptera), whose presence is limited exclusively to the dry season (December to April). The characteristics of the water quality, with large annual and seasonal variation to allow the re-colonization of organisms into Peje Stream, however, those organisms are incapable of developing and growth in this stream, providing evidence of a chemical barrier for the establishment of these species during the rest of the year. This investigation represents an approximation to the study of tolerance of tropical MI to the prolonged presence of pesticides and nutrients in water.
acetylcholinesterase (AChE), the content of non-protein thiols (NPTH) and the occurrence of lipid peroxidation (LPO) and protein carbonylation (PCO) were evaluated in different tissues. In addition, genotoxic biomarkers, such as DNA damage and the occurrence of micronuclei and nuclear erythrocytic change (ENAS), were analyzed in blood cells. The results of the biomarkers were integrated in the "Index of Biomarker Response" (IBR). Samples of water and sediments were collected from two sites in each period. Higher GST activity was observed in the liver and gills of fish caged in AP and in JC, respectively. It was also observed increased LPO in the liver of fish caged in AP and JC. On the other hand, lower AChE activity was observed in the brain and muscle of fish caged in AP and JC. These animals also showed higher scores of DNA damage and occurrence of ENAS in comparison with fish caged in GD. Several pesticides were found in water in concentrations exceeding the Brazilian guidelines, highlighting DDT, DDE, DDD, HCH, heptachlor, dichlofumid and aldrin. The AP stream showed the highest concentrations of the measured compounds, followed by JC and GD. According to IBR results (AP 21.7°C, JC 18.5°C, GD 12.6°C), and AP and JC streams showed the lowest environmental quality, corroborating the chemical analysis of the water and sediment, while GD showed a lower degree of contamination, probably related to the protection of the natural vegetation. By means of the integrated analysis of biomarkers, it appears that the changes in biomarkers correlated well with the levels of environmental contaminants, demonstrating that this approach is suitable for biomonitoring.

TH006 Assessing Pollution in Marine Protected Areas: The role of a multi-biomarker and multi-organ approach
P. Gusso Choueri, Universidade Estadual Paulista - UNESP, R.B. Choueri, Universidade Federal de São Paulo; G. Araújo, Universidade de Aveiro / Biologia; A.F. Cruz, Universidade Estadual Paulista - UNESP, T. Stremel, S. Campos, Universidade estadual de Ponta Grossa - UEPG, C.A. Ribeiro, Universidade Federal do Paraná; D. Abessa, Unesp / Marine Biology and Coastal Management

The role of multi-organ and multi-biomarker approaches in target tissues with metal burdens in muscle and liver tissues and PAH levels in the bile of C. spatula. Higher metal body burdens were quantified on liver and muscle (Cu, Mn, Zn, Cr, Co, Ni, Cd, Pb), and PAHs metabolites were identified in bile. The catfish (C. spatula) was collected from the local population and was used as a biomonitoring species. The results were integrated by the use of multivariate analysis. The use of the biomarker approach allowed for the identification of both seasonal and spatial variations in pollution sources around the APA-CIP. The current study shows that rainfall seasonality plays an important role in the estuarine pollution of the APA-CIP in terms of risks to the biota. The evidence includes the association of biomarker responses in target tissues with metal burdens in muscle and liver tissues and PAH levels in the bile of C. spatula. Higher metal body burdens associated with biological responses were found in sites under the influence of urban areas during the dry season, and they were found in the sites under the influence of the Ribeira de Iguape River (RIR) during the rainy season. The liver was found to be more responsive in terms of its antioxidant responses, whereas gills were found to be more affected by the effects of chemical pollutants. Such a study of biomarker analyses in different organs of fish is a useful tool for assessing chemical pollution in an MPA. This study adds to the knowledge on the effectiveness of MPA management. Such tools provide information for making sound decisions regarding environmental health status in vulnerable or valuable marine areas, and they support the management of MPAs and policies for conservation.

TH007 The role of a multi-biomarker approach in crab within an integrated sediment quality assessment in a Marine Protected Area
G. Araújo, Universidade de Aveiro / Biologia; P. Gusso Choueri, L.B. Moreira, Universidade Estadual Paulista - UNESP, D. Abessa, Unesp / Marine Biology and Coastal Management

Estuarine and marine Protected Areas (PAs) may be vulnerable to anthropic pressures, as the contaminants introduced through the continental drainage. This study aimed to evaluate if sediments from the Cananéia-Iguape-Peruíbe Protected Area (named APA-CIP) are contaminated by metals at levels that negatively affect the physical, chemical, and biological properties of the ecosystem. The obtained results indicated that the biomarker approach allowed for the identification of both seasonal and spatial variations in pollution sources around the APA-CIP. The current study shows that rainfall seasonality plays an important role in the estuarine pollution of the APA-CIP in terms of risks to the biota. The evidence includes the association of biomarker responses in target tissues with metal burdens in muscle and liver tissues and PAH levels in the bile of C. spatula. Higher metal body burdens associated with biological responses were found in sites under the influence of urban areas during the dry season, and they were found in the sites under the influence of the Ribeira de Iguape River (RIR) during the rainy season. The liver was found to be more responsive in terms of its antioxidant responses, whereas gills were found to be more affected by the effects of chemical pollutants. Such a study of biomarker analyses in different organs of fish is a useful tool for assessing chemical pollution in an MPA. This study adds to the knowledge on the effectiveness of MPA management. Such tools provide information for making sound decisions regarding environmental health status in vulnerable or valuable marine areas, and they support the management of MPAs and policies for conservation.

TH008 Biochemical and genotoxic biomarkers in the Neotropical fish Prochilodus lineatus caged in a river contaminated by tannery effluents

The objective of this study was to analyze biochemical and genotoxic biomarkers, as well as to determine chromium levels, in different tissues of the Neotropical fish Prochilodus lineatus submitted to in situ tests in Bandeirantes do Norte river (Parana State, southern Brazil), which receives tannery effluents. Juveniles of P. lineatus were submitted to in situ tests at four different river locations: site A – upstream of the tannery; site B – next to the tannery; and sites C and D – downstream of the tannery. After 96 h of caging, samples were collected from fish tissues, river water, and river sediments in order to quantify chromium levels. In addition, tissue samples were used to assess the activity of ethoxyresorufin–o-deethylase (EROD), glutathione S-transferase (GST), and acetylcholinesterase (AChE), the content of glutathione (GSH), metallothionein-like proteins (MT) and lipid peroxidation (LPO), and DNA damage levels by the comet assay. Higher chromium concentrations were detected in the water and sediments from site B and in the liver of fish confined at site B compared to the other locations. Fish caged at site B also showed higher levels of MT and EROD activities in their livers, in comparison to the fish caged at the other sites. Moreover, fish at site B had increased liver and branchial GST activities, as well as higher GSH concentration in the liver, than fish caged at site A. There were no statistically significant variations in LPO, DNA damage and brain or muscle AChE activity. These results indicated that tannery effluents increased chromium levels in the water, sediments, and fish livers and stimulated the synthesis of MT and GSH and the activities of EROD and GST. In conclusion, tannery effluents compromised the quality of the river water despite previous treatment, which resulted in altered biochemical markers and chromium accumulation in animals exposed to this river.

TH009 WATER AND SEDIMENT TOXICITY IDENTIFICATION AND EVALUATION OF A TROPICAL URBAN RESERVOIR
A.C. Rietzler, Departamento de Ciências Ambientais, Universidade Federal de Minas Gerais, Brazil; B. Lunardelli, M.T. Cabral, Ribeirão Preto / UNESP, C.B. Martins Ribeiro, Departamento de General Biology

Pampulha reservoir, a urban eutrophic system located in Belo Horizonte, Minas Gerais, Brazil, has been studied from a limnological point of view for many years, showing high inputs of domestic and industrial effluents from its tributaries. Ecotoxicological studies which began about 15 years ago, also showed acute and chronic toxicity effects of effluents on both aquatic and non-aquatic organisms. In recent years, an attempt to identify contaminants involved in water and sediment toxicity effects after being transferred to culture water, corroborating neurotoxic effects. Some biomarkers responses seemed to better respond to natural/seasonal variations in pollution sources and to environmental health status in vulnerable or valuable marine areas, and they support the management of MPAs and policies for conservation.

TH010 The joint effect of chlorpyrifos and fenobucarb on acetylcholinesterase in Daphnia similis
G. Gorini, Department of General Biology; F. Carriazo, Department of General Biology; C. Linnartz, Stockholm University / fisheri

The joint effect of chlorpyrifos and fenobucarb on acetylcholinesterase in Daphnia similis was evaluated. Samples of water and sediments were collected in June 2014 showed again water acute toxicity effects was made in 2011 and 2012 (TIE-PHASE I, USEPA, 1991), indicating non-polar organic and filterable compounds, as well as oxidant compounds as water contaminants and ammonia as sediment contaminant. Ecotoxicological studies which began about 15 years ago, also showed acute and chronic toxicity effects of water and sediments on Daphnia similis. This study indicated that tannery effluents increased chromium levels in the water, sediments, and fish livers and stimulated the synthesis of MT and GSH and the activities of EROD and GST. In conclusion, tannery effluents compromised the quality of the river water despite previous treatment, which resulted in altered biochemical markers and chromium accumulation in animals exposed to this river.

TH011 Climbing perch (Sicyopterus silvestrii) used as test organisms (ABNT 2004a; 2005a). More recently, an attempt to identify contaminants involved in water and sediment (interstitial water) acute toxicity effects was made in 2011 and 2012 (TIE-phase I, USEPA, 1991), indicating non-polar organic and filterable compounds, as well as oxidant compounds as water contaminants and ammonia as sediment contaminant. Focusing on the water TIE approach, preliminary toxicity assays with water samples collected in June 2014 showed again water acute toxic effects only on Daphnia similis. TIE experiments conducted afterwards, mainly indicated, again, effects of non-polar organic and filterable compounds, which were attributed to cyanobacteria cells and toxins. The specimens tested could recover from immobility effects after being transferred to culture water, corroborating neurotoxic effects of cyanobacteria (Cylindropermopsis raciborskii and possibly Planktothrix sp.) Besides, that sonification of filters containing water particulate matter organic and testing showed recovering of toxicity effects on Daphnia similis and Ceriodaphnia sp., which initially did not show water toxic effects. In this study, probably the broken cyanobacteria cells exposed even more toxins to the organisms tested, which effects were basically associated to ingestion by D.similis and not by C.similis.
of climbing perch has declined during the recent years. Organophosphates, such as chlorpyrifos, and carbamates, such as fenobucarb, are commonly used to control a wide range of pests in rice fields. These pesticides are highly toxic to aquatic organisms due to their ability to inactivate the enzyme acetylcholinesterase. This study assesses the combined effect of chlorpyrifos and fenobucarb on brain acetylcholinesterase in climbing perch fingerlings from rice field in the Mekong Delta. After exposure, the water concentrations of both pesticides were decreased quickly below detection levels within three days. However the inhibition of brain AChE activity continued for several more days. The result also indicates a quicker and less prolonged inhibition of brain AChE activity from the mixture than when the fish were exposed to only CPF.

TH011
Calculating herbicide sorption coefficients using selected soil properties in Brazilian tropical conditions
K.F. MENDES, Laboratory of Ecotoxicology; F.C. Reis, USP/CENA / Lab Ecotoxicologia; v. tassileio,
Herbicides play an important role in modern agriculture, but concerns about food safety and environmental impacts of herbicide residues have increased. Therefore, current mathematical models for predicting herbicide behavior in tropical soils use sorption coefficients (Kds) and organic carbon affinity (Koc) obtained from temperate soils, indicating that the information obtained may be distorted. Moreover, organic matter content in tropical soils varies greatly when methods and laboratories are compared; consequently, those conditions cause Kd values to vary. Thus, the objectives of this paper were to improve the scientific literature for evaluating Kd and Koc values of tropical soils using the values described in the databases for temperate soils, and correlate Kd values with tropical soil properties. Kd values were calculated based on an OC/OM (organic carbon/organic matter) index of 0.54 for 22 herbicides. Pearson correlation was used to compare Kd values to OC/OM and soil properties (organic matter, OM; clay mineral, CM; potential hydrogen, pH and cation exchange capacity, CEC) for each herbicide in tropical soils. The results indicate that Kd and Koc values for herbicides in tropical soils presented an OC/OM index ranging from 0.395 to 1.275. The Kd values of the majority of the herbicides correlate with the variation of OM and CEC contents in tropical soils. Research in Brazil is already generating information such as sorption coefficients, however, they are not tabulated in a database as has been done for temperate soils. Thus, it was possible to group the information related to Kd and Koc values of herbicides in tropical soil conditions. Standardized values confer more precision and accuracy to the mathematical models used when to represent herbicide behavior in soils with similar properties to tropical soils.

TH012
Quantification of oxadiazon residues in soils samples using a system solid-liquid extraction with low temperature partitioning by high performance liquid chromatography
K.F. MENDES, Laboratory of Ecotoxicology; M.R. REIS, UFV / Agricultural SCIENCES INSTITUTE; v. tassileio,
Herbicides are increasing the productivity of modern agriculture. However, they have caused concern regarding food security and the environmental impacts of herbicide residues. Surface and subterranean waters have been contaminated by agricultural and non-agricultural herbicide applications, as a result of the physio-chemical characteristics of the soil and of the herbicide. Adding organic residues to agricultural soils is a widely used practice to increase organic matter content and prolong herbicide persistence. However, half-lives often determined in laboratory studies using disturbed soils, where the aggregates of the soil, micropores, macropores and the proportion of solution to solids differ from field soil conditions. The behavior of oxadiazon (5-tet-butyl-3-(2,4-dichloro-5-isoproxyphenyl)-1,3,4-oxidiazolid-2 (3H-one) in the soil may be influenced by organic matter content. The objective of this study was to determine the oxadiazon residues by chromatography as a function of organic material and soil depth, with Oxisol, clay texture, in Rio Paranaíba, Minas Gerais State, Brazil. A randomized block design was used, with treatments arranged in a scheme of 2 x 4 x 8 + 1 subdivided plots with four repetitions. The plots were comprised of soil with organic matter incorporated (8 t ha$^{-1}$) and without organic material incorporated, and a control (0 t ha$^{-1}$ and 0 g ha$^{-1}$ of oxadiazon). The subplots were the soil depths (0.00-0.05; 0.05-0.10; 0.10-0.15 and 0.15-0.20 m) and the subsubplots were soil collection time (0, 2, 4, 8, 16, 32, 64 and 128 days after application - DAA). Oxadiazon was applied at 1 kg ha$^{-1}$. For residue determination, samples were quantified with high performance liquid chromatography (HPLC) using the technique of solid-liquid extraction with low temperature partitioning (SL-PLP). The results indicated of 0.00-0.05 m, dissipation t$\text{1/2}$ of oxadiazon was between 56 and 51 days in soil with and without organic material incorporated, respectively. Oxadiazon was found in the upper layer (0.00-0.10 m) of soil until 64 DAA and did not occur at the other depths, demonstrating low soil mobility potential. In soils incorporated with organic material, higher concentrations of oxadiazon were observed over different depths and evaluation periods.

TH013
Evaluation on the joint toxicity of Chlorpyrifos ethyl and Fenobucarb on cholinesterase activity in Climbing perch fish (Anabas testudineus, Bloch, 1792) from rice paddy in the Mekong Delta, Vietnam
T.T. Nguyen Thanh, Stockholm University / fisheries; H. Berg, Orebro University; N.H. Pham, C.V. Nguyen, Can Tho University / College of Environment and Natural Resources
Abstract Climbing perch (Anabas testudineus, Bloch, 1792), is an important food fish to local people in the Mekong Delta. Although no official statistic, the wild population of climbing perch has declined during the recent years. Organophosphates, such as chlorpyrifos, and fenobucarb, are commonly used to control a wide range of pests in rice fields. These pesticides are highly toxic to aquatic organisms due to their ability to inactivate the enzyme acetylcholinesterase. This study assesses the combined effect of chlorpyrifos and fenobucarb on brain acetylcholinesterase in climbing perch fingerlings from rice field in the Mekong Delta. After spraying the, the water concentrations of both of pesticides were decreased quickly below detection levels within three days. However the inhibition of brain AChE activity continued for several more days. The result also indicates a quicker and less prolonged inhibition of brain AChE activity from the mixture than when the fish were exposed to only CPF. Keywords: Climbing perch, Chlorpyrifos; Fenobucarb; acetylcholinesterase

TH014
Is rice a suitable test organism in sediment-contact bioassays for the assessment of sediment toxicity?
A. Brinke, German Federal Institute of Hydrology; G. Reifferscheid, Biochemistry and Ecotoxicology; R. Klein, Trier University; S. Buchinger, Federal Institute of Hydrology / Department G Biochemistry Ecotoxicology; U. Feiler, Federal Institute of Hydrology
Rice is the staple food for 50% of human population. Most rice breeding is done in sub- and tropical regions. Today, ecotoxicological research on rice focuses on toxic compounds in contaminated paddy field soils, which are already a threat for rice farmers and consumers. Pollutants are heavy metals and arsenic (mainly geogenic, but also from anthropogenic origin) as well as organic pollutants (from pesticides, air transport, etc.). The development of suitable tools for an adequate risk assessment on rice fields is consequently highly required. Up to now, only ecotoxicological bioassays with rice focus on aquatic in vivo testing. However, sediment-contact bioassays provide a more realistic scenario for the ecosystem functions of sediments and hazards arising from bioavailable sediment-bound contaminants. They are therefore a more suitable method to deploy interactions between sediment and test organism. This study implies a sediment-contact bioassay for the assessment of sediment toxicity in tropical regions with the rice plant Oryza sativa. Rice was exposed on arsenic, cadmium, chromium and nickel spiked sediments. The classic endpoints inhibition of root and shoot elongation were evaluated as sensitive endpoints for sediment toxicity in single-substance tests on artificial sediment. Rice was proved to be most sensitive than shoots, which is displayed in the determined EC$_{50}$ [Shoots: EC$_{50}$(As)I=24 mg kg$^{-1}$; EC$_{50}$(Cr)I=373 mg kg$^{-1}$; EC$_{50}$(Cd)I=2032 mg kg$^{-1}$; EC$_{50}$(Ni)I=600 mg kg$^{-1}$; EC$_{50}$(Cd)II=477 mg kg$^{-1}$; rats: EC$_{50}$(As)II=13 mg kg$^{-1}$; EC$_{50}$(Cr)II=334 mg kg$^{-1}$); EC$_{50}$(Cd)II=1288 mg kg$^{-1}$; EC$_{50}$(Ni)II=218 mg kg$^{-1}$; EC$_{50}$(Cd)I=408 mg kg$^{-1}$]. On natural sediments these endpoints did not prove to be enough sensitive. Observations were made concerning dose-dependant changes in root morphology which could not be assessed by classical root endpoints and are therefore implied as new toxicity endpoints. Characteristic changes were e.g. the loss or shortening of lateral roots. Rice showed sediment-bound pollutant induced changes in growth and is therefore a possible test organism for sediment-contact bioassays with higher plants. However, classic root endpoints are not sensitive on natural sediments. New endpoints for bioassays with higher plants could be qualitative endpoints but also alternative quantitative endpoints, concerning morphological changes of the root system.

TH015
Acute toxicity testing of two reference toxicants on a copepod species (Euterpina acutifrons) common in Qatar waterways
N. Deb, ExxonMobil Research Qatar / Environmental; S. Saeed, ExxonMobil Research Qatar; S. Bacha, Environmental Studies Center / Technical; E. Marquis, A. Grimm, URS Qatar LLC
Bioassay of several reference toxicants on phytoplankton and fish embryos native to the Arabian Gulf, a culture of this species was successfully established in the Exxomobil Research Qatar laboratory to derive environmental quality bio-monitoring and subsequent risk evaluation of potential contaminants in the region. To obtain a more toxicologically comprehensive and ecologically representative picture of the marine ecosystem, toxicity tests were initiated on copepods. They are a major link between the primary producers and heterotrophic consumers in the marine food webs. Furthermore, marine copepods, such as Euterpina acutifrons, a common species in Qatar waters, have been shown to be nutritionally superior (rich in highly unsaturated fatty acids) live feed in aquaculture, an industry developing fast in Qatar. Euterpina acutifrons is a sexually dimorphic, pelagic, harpacticoid copepod distributed worldwide, including the Arabian Gulf. A culture of this species was successfully established in the laboratory from one gravid female obtained from the Qatari coast. The highest survival and fecundity of the species were obtained at 22 ± 2°C under a 12 h light :12 h dark light regime, in cultures maintained in artificial sea water at 40 ± 2 ppt salinity, and fed with a microalgal mixture of Chaetoceros sp. and Synecococcus sp. The present study evaluated in a static set-up, the acute toxicity of two reference
toxicants, sodium dodecyl sulfate (SDS) and 3, 4-dichloroaniline (DCA) on laboratory cultured Euperna acutifrons. The test end point was the immobilization or reduced mobility of the copepods after 24 and 48 hours. Based on preliminary studies, different range concentrations were chosen for the two toxicants. The results show that SDS, an anionic surfactant, has a more pronounced and faster impact than DCA, on the immobilization of this species. This can be attributed to the different mechanism of action of these two toxicants. Our results suggest that low concentrations of SDS can critically disrupt the marine food web by causing a decline in the copepod density within a short time. The widespread distribution, ecological importance in bottom-up and top-down control in the food web, feasibility of laboratory culturing, continuous egg production throughout the year, and short life-cycle render this copepod to be an ideal species for conducting acute and chronic life table tests for water-borne contaminants found in the Gulf of Carabobo.

Pesticides in air in a banana production area at the Caribbean coast of Costa Rica

L. Cordoba, Universidad Nacional / Central American Institute for Studies on Toxic Substances IRET; K. Solano, C. Ruiperez, Universidad Nacional / IRET; R. van Wendel de Joode, Universidad Nacional / Central American Institute for Studies on Toxic Substances IRET

Agricultural activities in Costa Rica are characterized by intensive soil use and depend on a high input of agrochemicals due to the climate. Weekly aerial spraying of pesticides in a commercial banana plantation in Costa Rica, even though often situated very close to villages. Nematicides and herbicides are applied by ground application and banana bunches are protected with insecticide-treated bags. Environmental exposure to pesticides can cause ecological and human health risks. As a part of a large research project called Infants’ Environmental Health program (ISAin which exposure and related health effects are assessed in banana producing regions of Costa Rica[1], different sampling techniques were applied for evaluating environmental air concentrations of current use pesticides in the Matina county located at the Caribbean coast. Air samples were collected for six months using passive and active sampling techniques based on polyurethane foam disks (PAS-PUF) and high volume air sampler with particle collection and XAD- foam cartridges (AAS-XAD). Pesticide deposition was collected by petri dishes. The PAS-PUF samplers were located at primary schools in 12 villages of the Matina county, 10 immersed in banana plantations and 2 located at about 2 kilometers’ distance. The PUF-disks were deployed for exposure periods of 27-55 days at each site and were changed 4 times between June and December 2010; a total of 51 air samples were collected. Sampling rates for the particulate air samplers were determined by the loss of spiked depuration compounds. The 24 hour AAS-XAD samples were taken at 4 schools, at the start and end of each PAS-PUF sampling. The insecticide chlorpyrifos was detected in all air samples with a mean of 11.2 ng.m/3, for immersed schools six times higher concentrations were found as compared to referent schools. The other most frequent detected pesticides for both sampling techniques were the fungicide chlorothalonil and fenpropimorph, and the nematicides cadusafos, ethopropos and terbufos. The fungicides epoxiconazole, spiroxamine and difenoconazole were detected mainly in the particulate fraction of the AAS-XAD. This behavior was confirmed by the deposition on petri dishes. Passive air samplers can be effectively used to measure average concentrations of pesticides at low levels in a tropical agricultural area. ‘n [1] ISA: www.isa.una.ac.cr

Correlations between metallothionein, metals and fish morphometric indices indicative of environmental contamination in an antropically impacted estuarine lagoon in Rio de Janeiro, Brazil

s_a.santos, Biodiversity PostGraduate Program; T. SaintPierre, Puc-Rio; R.A. Hauser-Davis, Biodiversity PostGraduate Program; R.L. Zioli, UNIRIO

Of all the environmental pollutants, metals are of particular concern due to their potential toxic effects and their ability to bioaccumulate in aquatic ecosystems. Metallothioneins (MT) are a validated biomarker for metal exposure in aquatic organisms. DDTP is a fish liver contaminant, since it is the main detoxifying organ. However, recent studies indicate that metals and MT excreted in bile can also be used as biomarkers in this context. Several reports also relate metal contamination to changes in morphological parameters that describe environmental air concentrations, indicating that the presence of Ni in these tissues could induce metallothionein synthesis. We also observed that Ni exposure affected antioxidiant defenses, increasing lipid peroxidation in the liver of fish exposed to Ni for 96 h at the highest concentration tested. DNA damage increased in both blood cells and gills of fish exposed to all Ni concentrations, indicating the genotoxic potential of Ni to fish. We therefore concluded that Ni accumulates in the liver and gills after exposure to Ni, implying that the presence of Ni in these tissues could induce metallothionein synthesis. We also observed that Ni exposure affected antioxidiant defenses, increasing lipid peroxidation in the liver of fish exposed to Ni for 96 h at the highest concentration tested. DNA damage increased in both blood cells and gills of fish exposed to all Ni concentrations, indicating the genotoxic potential of Ni to fish. We therefore concluded that Ni accumulates in various tissues and promotes oxidative and DNA damage in P. lineatus and that the maximum permitted Ni concentration in Brazilian natural freshwaters is not safe for this fish species.

Occurrence and possible impact on the aquatic environment of drugs of abuse in a Central American country as established from sewage water analysis A. Cauanasllie, J. Sibaja, Universidad Nacional / IRET; E. Emke, KWR Watercycle Research Institute; C. Rueda y Alfonso, Universidad Nacional / IRET; F. de Voogt, University of Amsterdam / IBED

Chemical analysis of waste water entering sewage treatment plants (STPs) can provide information on the use of illicit drugs in the population connected to the sewer system. In recent years several studies have been published in Europe in which the daily or weekly loads of drugs of abuse transported to municipal STPs has been determined and of these studies that assessing the potential of these compounds to be found in wastewater. In addition it has been shown that several of these compounds are not removed by the treatment, resulting in actual discharges to surface waters. The present study was conducted in Costa Rica with the aim of collecting data on occurrence of illicit drugs in wastewater and surface waters in this country and to compare these levels with known data from Europe. To that end samples from two different STPs that used completely different treatment processes were collected, together with samples from nearby surface waters. In 2012 and 2014 grab samples were collected, whereas in 2014 also seven 24 composite samples of influents from each of the two STPs were collected. The concentrations of the illicit drugs were determined by an analytical procedure that involved filtration, solid phase extraction and GC/MS. The results showed the presence of several illicit drugs in the influents collected, including cocaine, benzoylcegonine, THC-COOH and codeine in the µg/L range and ritalinic acid and mephedrone in the ng/L range.

Toxic effect of detergents in organisms of different trophic levels

A.S. Sohrinio-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia Laboratorio Alejandro Villalobos

In this study an evaluation of the toxicity of 2 surfactants (alkyl lauryl sulfonate (LAS) and lauryl dimethil (hidroxietil) ammonium chloride) and 4 trademarks of detergent (Ariel, Foca, Roma and Salvo) was carried out in microalgae (Pseudokirchneriella subcapitata), Monoraphidium illalobos, Monoraphidium illalobos, macrophytes (Lemna gibba and Egeria densa), cladocerans (Daphnia magna, D. exilis, Moina macrocephala and Simocephalus mystax), ostracods (Cyprip sp.) and fish (Danio rerio) due to in our country studies with these compounds are scarce. Toxicity bioassays were carried out with duration of 48 hours for tests with cladocerans and fish...
ostracods, and 96 hours for tests with microalgae, macrophytes and fish. The organisms were exposed to five concentrations of each of the detergents in triplicate (0.1, 1.0, 10 and 50 and/or 100, 200, 400, 800 and 1000 mg L⁻¹) plus one control, without toxic. Each test at least three times was repeated. With the data obtained lethal concentration 50 (LC₅₀) was determined by probit method (Probit-EPA, version 1.5) and a comparison between the LC₅₀ and confidence intervals was made to evaluate the statistical significance of the difference between the different treatments with detergents. The probit tests were performed to obtain significant differences in the response of species to detergents, P. subcapitata, Monoraphidium sp, D. exilis and Cypria sp. were the most sensitive species and macrophytes Lemna gibba and Egeria densa were less sensitive to these products. The toxicity of detergents containing enzymes was superior compared with detergents not have enzymes in its formulation. Due to the wastewater treatment is limited and often sewage with high concentrations of detergents are discharged directly to aquatic systems, it is important to know the potentially adverse effects of these compounds to propose appropriate measures to reduce the risk which involves their presence in aquatic environments.

TH021
Oxidative damage in gills and kidney and antioxidant defense system of traíra, Hoplias malabaricus, injected with crude extract of cyanobacteria, Radiocystis fernandoi. J.B. Fernandes, M.M. Sakuragui, Federal University of Sao Carlos / Department of Physiological Ciencies; N.E. da Silva de Souza, T. Peixoto, M.G. Paulino, D. Tavares, Federal University of Sao Carlos / Department of Physiological Sciences; A.P. Trechman, J.B. Fernandes, Universidade Federal de Sao Carlos / Quimica; A. Giani, Federal University of Minas Gerais / Department of Botanical; M.N. Fernandes, Universidade Federal de Sao Carlos / Ciencias Fisiologicas

The microcystins (MCs) are toxins produced by cyanobacteria that may increase the free radicals and induce oxidative stress in different tissues of fish. In this context, this study evaluated the toxicity of MCs present in the crude extract of Radiocystis fernandoi on the gills and kidney of traíra, Hoplias malabaricus. Fish were separated in control group (SA30D, injection with saline 0.9%) and MC group (MC30D, intraperitoneal injected with MCs (100 μg kg⁻¹ body mass) every 72h during 30 days. After that the gills and kidney were sampled and the activity of ethoxyresorufin O-deethylase (EROD), superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST), glutathione peroxidase (GPX) as well as the levels of glutathione (GSH) and lipid peroxidation (LPO) were determined. No significant changes occurred in activities of EROD, SOD and GPX and in the levels of GSH and LPO of gills. However, the activity of GST increased in the gills. In the kidney, the EROD activity and GSH levels decreased, the activities of SOD, CAT and GST catalase were increased. GST catalase activity showed conjugation of xenobiotes with GSH, thereby facilitating their elimination from the cell. The increased activities of GST in the gills after prolonged exposures to MC suggested biotransformation responses. The increased activities of SOD, CAT and GPX and levels of LPO in the kidney indicate that the antioxidant responses was not efficient to avoid oxidative stress in the MC group injected with crude extract of R. fernandoi injection. The new antioxidant treatment will be developed to reduce the effects of MCs contamination. Financial support: CAPES Proc. 2276/2011, CEMIQ Proc. GT346 and CNPq/INCT-TA Proc. 573949/2008-5. LPO- gills, kidney

TH025
Environmental impact of plant protection in the Norwegian catchment Skuterud analysed with the GIS-based indicator SYNOPS J. Strømsøyever, ral Research Centre for Cultivated Plants / Institute for Strategies and Technology Assessment; O.M. Eklo, M. Stennd, Bioforsk - Norwegian Institute of Bioeconomy Research; E. Solbakken, R. Liegau, Norwegian Forest and Landscape Institute

The use of pesticides in agriculture causes environmental risks that must be carefully managed. On EU level various Directives and National Action Plans have set reduction of agriculture-related pollution on the agenda. To fulfil this goal the Norwegian Project STRAPP focusses to develop instruments aiming at the identification and mitigation of new active substance. This proactive approach is part of the Norwegian agricultural environmental monitoring programme. It is located approximately 30 km southeast of Oslo. The total area of the catchment is 450 ha and main land use is agriculture with a total area of 272 ha and 51 individual farmer fields. Since 1993, the farmers have provided field specific information about farming practices. For this catchment the risk indicator model SYNOPS-GIS was applied considering the spatial distribution of the surveyed spray schedules together with an extended GIS based datasets on land use, slope, soil types and climate. Furthermore a database on pesticides was linked giving information on formulation, active ingredient (a.i.) content and product specific drift and run-off mitigation requirements. SYNOPS-GIS assesses the risk of chemical plant protection products for terrestrial and aquatic organisms. It combines use data of pesticides with their application conditions and their inherent properties. The predicted environmental concentration of a.i.’s is calculated on a daily basis for soil, surface waters and off field areas considering crop interception, spray drift, surface run-off and drainage, temperature-dependent degradation in water and soil. From the daily environmental concentrations the risk potentials are calculated as the exceedance probability of concentration specific species sensitive to pesticides for terrestrial and aquatic species. Assessments of the aquatic risk were conducted on field level and annual basis since 2004. The calculated risks were aggregated on catchment level and risk-trends could be demonstrated. A clear decline of the risk since 2004 could not be shown. Crop specific analysis within the catchment showed that winter wheat, barley and spring wheat were at a similar risk level showing low 95th percentiles of the aquatic risk with values of 0.05 for winter wheat, 0.1 for barley, 0.03 for oats and 0.08 for spray. It is planned to include possible risk mitigation measures in the SYNOPS assessments in order to demonstrate the impact of the measure on catchment level.

TH024
Antibiotic resistance and bacterial community changes in a gradient from sea cage aquaculture in central Vietnam M. Nitz Peterssson, Stockholm University / EMB; N. Hedberg, D. Warshan, Stockholm University / Department of Ecology Environment and Plant Sciences; M. Tengdren, Department of Ecology Environment and Plant Sciences DEEP Antibiotics are intensively used in the Vietnamese aquaculture industry in order to prevent or treat diseases affecting the cultivated fish and lobster. The small-scale family owned farms dominate the sea cage aquaculture industry and are often found close to coral reefs, providing the cages with services such as clear water and shelter from waves. Overuse of antibiotics affects the microbial community present within the coral by creating antibiotic resistant bacteria, adding a selective pressure that changes the composition of the coral associated bacteria. The coral microbial community has shown to be important for corals, serving as an immune system supporting their resilience to external pressure. In this study we investigate the resistance capacity of bacteria associated with the solitary free-living coral Fungia fungites sampled within the Nha Trang Bay area in south-central Vietnam. The antibiotics used in the survey are the three most commonly used in aquaculture in the area (Tetracycline, Rifampicin and Vancomycin) and the antibiotic resistance is assayed in a distance gradient with sites located at 50 m up to 2700 m away from the sea cages. The 16S rRNA gene of 40 bacteria from 8 sites along the distance gradient were isolated and sequenced. Antibiotic resistance test were performed on similar strains from Barculus pumilus and B. niabensis found at different sites along the distance gradient. B. pumilus and B. niabensis showed antibiotic resistance at sites located up to 660 m away from sea cages when compared to the more pristine site at 2700 m. The resistance to antibiotics further showed to gradually increase from 50 m up to 2700 m away. It has been shown that the antibiotic use does affect the coral associated bacteria found close to the sea cage aquaculture activities. This might affect the immune system function provided for the coral, which might give increased sensitivity to coral diseases. It has further been shown that antibiotics can reduce the settling success among coral larvae, indicating that the antibiotics used in aquaculture may have an impact not only on the bacteria associated with corals but also on several different species and functions found within the coral reef ecosystem. The antibiotic use is one of many pressures added and must be taken in to consideration with i.e. higher nutrient levels in the water caused by the intensive feeding at the farms when investigating the coral reef resilience.

TH023
Framework to establish water quality criteria for livestock in Brazil G. Boer de Souza, SCHOOL OF TECHNOLOGY UNICAMP / LEAL; Laboratory of Ecotoxicology and Environmental Microbiology; D. SPRY, ENVIRONMENTAL CANADA; J. Palhares, Embrancha; L. Rudez, Subsecretaria de Recursos Hidricos; G.d. Umbuzeiro, FACULTY OF TECHNOLOGY -UNICAMP / LEAL

Water quality standards of the Brazilian regulations are based on international or other countries data, which differ from Brazil among exposure scenarios and public management policies. On the other hand, contradictions among quality standards in force for the same water uses and the lack of transparency with respect to establishment of these criteria can cause conflicts and inappropriate water resources management. Protocols for deriving water quality criteria for drinking water and protection of aquatic life have already been developed (www.http://mutagen-brasil.org.br/documentos/). However, there are some gaps regarding other uses. Cattlearing activity is the single most important contributor to Brazilian economy.Good water quality for livestock is essential to preserve the quality assurance of water for animals and humans. For full protection of aquatic life have already been developed framework to establish water quality criteria for livestock in Brazil among exposure scenarios and public management policies. On the other hand, contradictions among quality standards in force for the same water uses and the lack of transparency with respect to establishment of these criteria can cause conflicts and inappropriate water resources management. Protocols for deriving water quality criteria for drinking water and protection of aquatic life have already been developed (www.http://mutagen-brasil.org.br/documentos/). However, there are some gaps regarding other uses. Cattlearing activity is the single most important contributor to Brazilian economy.
Pentachloroanisole is not a relevant proxy for pentachlorophenol in the Arctic environment

H. Kylin, Linköping University / Department of Thematic Studies - Environmental Change; H. Bouwman, North-West University / Environmental Sciences and Development

Pentachlorophenol (PCP) is currently under evaluation for inclusion in the Stockholm Convention (SC). Unfortunately, there are few data on PCP in environmental samples, largely because PCP, as an ionisable compound, cannot be included in standard analytical protocols for POPs. Often, PCP is determined after derivatization to pentachloroanisole (PCA) which can be determined together with other POPs. However, PCP is also undergoes microbial methylation to PCA in the environment, and as no other sources of PCA are known it has been assumed that PCA can be used as proxy for PCP in the environment, not least the Arctic. A further complication interpreting environmental data is that PCP and PCA, to save time, often are determined together as derivatization of PCP to PCA often is done without prior separation of PCP from the PCA native to the sample. This procedure also presupposes that PCA is a functioning proxy for PCP. Compiling data from several sampling series of pine needle samples from low latitudes to the Arctic shows that PCA does not function as proxy for PCP. While PCP concentrations as expected are highest at locations where it was still in use, PCA has a northern marine signature; the highest concentration in Europe are found along the coasts of the Norwegian and Barents’ Seas with lower concentrations in continental locations at similar latitudes. Regionally, the PCA concentrations are elevated at PCP hot spots or used to seed treatment may end up into aquatic systems. In aquatic systems they can be harmful for other biota as well because they have been originally developed as poisonous to the target biota. We used solid phase extraction coupled with mass spectrometric gas chromatography (GC/MS/MS) and ultra performance liquid chromatography (UPLC/MS/MS) to analyze the concentrations of the POP active substances, their breakdown products and other biocides in surface water samples. We compared the concentrations to environmental quality standards (EQS) and to corresponding reference values (RV) to yield risk quotients for contamination risk assessment. Our data correspond to years 2007-2012. Plant protectants were observed in both lotic and lentic waters under the influence of agriculture. Altogether 68 various compounds were observed from the 201 analyzed ones. The most frequently found compounds were phenoxy acid herbicides. Methyl chlorophenoxy acetic acid (MCPA) was the most abundant herbicide observed but its concentrations were below the nationally set EQS during the whole observation period. Ten of the observed compounds exceeded the EQS or RVs at least once whereas the quantification limits of seven other compounds were higher than the EQSs or RVs. Of these ten compounds four were low dose harmful agents: metolachlor (2008), metribuzin (2010), thifensulfuron (2008) and thifensulfuron (2011), originated from bioide usage. Two of the compounds, furalactar (2009) and malathion (2010) were used as insecticides. The origin of individual peak concentrations of highly toxic organochlorine insecticides endosulfan and endrin (2009) binned in many countries remained unclear. Our monitoring data and environmental quality comparisons revealed traces of POPs cause no extensive harmful effects in Finnish freshwaters during our observation period. Individual peak concentrations, however, demonstrated the need for continuing monitoring. Sampling sites and intensities were sparse taking into consideration the typical complex river-lake networks of thousands of lakes in Finland.

TH026

Does the discovery of a new blue mussel species in Greenland have implications for its use in monitoring programs?

L. Bach, Aarhus University (AU), Arctic Research Centre / Department of Bioscience; J. Strand; G. Asmund, Aarhus University / Department of Bioscience

In Greenland, we have so far assumed that there was only one species of blue mussels, Mytilus edulis. A brand new study, have now shown that there actually are two species: Mytilus edulis and Mytilus trossulus. The range of the two species in Greenland is unknown. As a result we do not know whether our previous monitoring programs have been based on one, the other or possibly a mix of the two species. A new study investigates the impacts of this discovery on monitoring programs. We study whether there are differences in ability between the two species to accumulate contaminants incl. metals from the Greenland environment. Studies of the uptake and accumulation of metals in mussels collected elsewhere in the world show indications that there may be such a difference. In Greenland there is a long history of using mussels as indicator organisms for environmental impact, both as monitoring program to assess contamination of the marine areas of especially of Pb and Zn. In order to shed light on the present contamination and possible effects in the fjord we initiated a range of studies including gill and liver morphology of common mussels (Mytilus edulis). The range of the species might vary but its concentrations were below the nationally set EQS during the two species to accumulate contaminants incl. metals from the Greenland environment. Two of the compounds, furatiokarb (2009), originated from bioside usage. Two of the compounds, metribuzin (2010), originated from bioide usage. Two of the compounds, furalactar (2009) and malathion (2010) were used as insecticides. The origin of individual peak concentrations of highly toxic organochlorine insecticides endosulfan and endrin (2009) binned in many countries remained unclear. Our monitoring data and environmental quality comparisons revealed traces of POPs cause no extensive harmful effects in Finnish freshwaters during our observation period. Individual peak concentrations, however, demonstrated the need for continuing monitoring. Sampling sites and intensities were sparse taking into consideration the typical complex river-lake networks of thousands of lakes in Finland.

TH027

Concentrations and risks of plant protection products for biota in Finnish freshwaters

A. Aallon, ECHA-European Chemicals Agency; K. Siimes, Finnish Environment Institute (SYKE); A. Aallon, Ramboll Analytics Oy / Niemenkatu Lahti; J. Mannio, Finnish Environment Institute SYKE / Centre for Sustainable Consumption and Production

Plant protection products (PPP) are used to maximize crop yields by protecting crop against pests, weeds and plant diseases and as growth retardants. PPPs spread to land or used to seed treatment may end up into aquatic systems. In aquatic systems they can be harmful for other biota as well because they have been originally developed as poisonous to the target biota. We used solid phase extraction coupled with mass spectrometric gas chromatography (GC/MS/MS) and ultra performance liquid chromatography (UPLC/MS/MS) to analyze the concentrations of the PPP active substances, their breakdown products and other biocides in surface water samples. We compared the concentrations to environmental quality standards (EQS) and to corresponding reference values (RV) to yield risk quotients for contamination risk assessment. Our data correspond to years 2007-2012. Plant protectants were observed in both lotic and lentic waters under the influence of agriculture. Altogether 68 various compounds were observed from the 201 analyzed ones. The most frequently found compounds were phenoxy acid herbicides. Methyl chlorophenoxy acetic acid (MCPA) was the most abundant herbicide observed but its concentrations were below the nationally set EQS during the whole observation period. Ten of the observed compounds exceeded the EQS or RVs at least once whereas the quantification limits of seven other compounds were higher than the EQSs or RVs. Of these ten compounds four were low dose harmful agents: metolachlor (2008), metribuzin (2010), thifensulfuron (2008) and thifensulfuron (2011), originated from bioide usage. Two of the compounds, furalactar (2009) and malathion (2010) were used as insecticides. The origin of individual peak concentrations of highly toxic organochlorine insecticides endosulfan and endrin (2009) binned in many countries remained unclear. Our monitoring data and environmental quality comparisons revealed traces of POPs cause no extensive harmful effects in Finnish freshwaters during our observation period. Individual peak concentrations, however, demonstrated the need for continuing monitoring. Sampling sites and intensities were sparse taking into consideration the typical complex river-lake networks of thousands of lakes in Finland.

TH028

The use of common scallops (Myxospocelus scopus) as a biomonitoring organism for biogenic indicators of mining activities in Greenland

R.D. Nørgaard, Aarhus University, DMU / DCE; L. Bach, Aarhus University (AU), Arctic Research Centre / Department of Bioscience; C. Sonne, Aarhus University / Department of Arctic Environment

The former mining areas Black Angel Pb-Zn mine in Maarmorilik (West Greenland) and Mestersvig Pb mine (East Greenland) have caused significant metal contamination of the marine environment in especially of Pb and Zn. In order to shed light on the present contamination and possible effects in the fjord we initiated a range of studies including gill and liver morphology of common scallops (Myxospocelus scopus). Scallops were caught and sampled at different stations known to represent gradients of low, intermediate and high Pb concentrations. Chronic lesions were observed in liver and gills which were ascribed to metal contamination of the mussel and lead concentrations of eluted Zn. The lesions were similar to those in literature studies for both wild and laboratory exposed scallops and other fish species. We suggest that beside exposure to mining-related elements, other environmental factors such as parasites are co-factors in the development of the observed liver and gill lesions. Therefore, scallop liver and gill pathology is
likely to be suitable health indicators when biomonitoring gradients of mining activity effects; while a larger study is required to fully evaluate the relationships.

TH031 Plants influence the deposition of POPs to remote terrestrial ecosystems

H. Kylin, Linköping University / Department of Thematic Studies Environmental Change; H. Bournan, North-West University / School of Environmental Sciences and Public Health

“Plants” have gained interest for biomonitoring of airborne POPs as they are present in most of the world making sampling logistics simple. Therefore, “plants” have been used to study global distillation or orographic cold trapping. To interpret the data it is essential to understand the uptake process. However, “plants” and “single phylogenetic entity”. Thus, using “plant” data to show global or orographic distillation or deposition to remote areas is complex; different “plant” taxa take up airborne POPs by different mechanisms. A basic difference is that homohydric vascular plants have hydrophobic surfaces, while poikilohydric mosses and lichens have hydrophilic surfaces. The volatile POPs that are present mostly in the gas phase, e.g. HCHs, show the largest discrepancies between models and measurements. In spite of different seasonal air concentration profiles of α-HCH and γ-HCH, the seasonal accumulation patterns in pine needles are similar, indicating that the uptake patterns are governed by biological, not physical-chemical processes. The seasonality of the uptake patterns strongly follow the amount of endogenous VOCs, e.g. terpenes, in the pine needles. Calculation of POP concentrations in vascular plants should not be done on a lipid mass basis; much of the hydrophobic oil content in the plants, “lipids”, will not be accounted for in the lipid determination. VOCs, e.g. will evaporate during the process, and the cutin of the cuticular membrane is a polymeric lipid that will not be extracted. The uptake of airborne HCHs in poikilohydric mosses and lichens occurs by an entirely different mechanisms than in homohydric vascular plants; uptake in mosses and lichens is governed by their hydration state. Fully hydrated both moss and lichen contain high amounts of HCHs when desiccated. Again, lipid determination is meaningless. Because of these factors, studies purporting to show global distillation or cold condensation by using “plant” data are questionable and need further clarification of biological factors involved. The temperature effect on plant biology indicates that more POPs will be deposited to terrestrial ecosystems with higher temperatures rather than the opposite. Climate change with longer vegetation periods will lead to more efficient scavenging of airborne POPs in boreal zone.

TH032 Biomarker modulation associated with marine diesel contamination in Icelandic scallops (Chlamys islandica): investigating the impact of an emerging pollution in the Arctic

P. Geraudie, Akvaplan-niva AS / Petroleum and environmental group; R. Bakkeno, Essex University; T. Milinkovitch, University of La Rochelle; h. thomas, The Arctic sea ice cover decline, due to ongoing climate change, will ineluctably result in the intensification of human activities in polar areas, including shipping activities, tourism, and exploitation of the resources. The development and application of biomarkers will contribute to a better understanding of the biological effects of marine diesel in Icelandic scallops (Chlamys islandica), a key benthic species from Arctic and subarctic ecosystems. Using a seven-day contamination protocol with three environmentally relevant concentrations of marine diesel and a control, a suite of biomarkers related to antioxidant response, neurotoxic effects, gonad maturation and lipid content was investigated.. A strong inhibition of muscular acetylcholinesterase activities was observed in contaminated Icelandic scallops while a significant induction of the biological effects of marine diesel in Icelandic scallops (Chlamys islandica), a key benthic species from Arctic and subarctic ecosystems. Using a seven-day contamination protocol with three environmentally relevant concentrations of marine diesel and a control, a suite of biomarkers related to antioxidant response, neurotoxic effects, gonad maturation and lipid content was investigated. Significant amounts of HCHs including marine diesel are released into the Barents Sea due to shipping activities, offshore oil and gas exploration, tourism and fishery. As only ma...
TH036 Artificial sunlight photolysis of the pharmaceuticals doxepin hydrochloride, gabapentin and quetiapine hemifumarate as single compounds and as a mixture
M. Herrmann, Institut für Umweltwissenschaften und Naturschutz, University of Lüneburg; C. Metzger, University of Lüneburg / Institute of Sustainable and Environmental Chemistry; O. Ölsön, Leuphana Universität Lüneburg; C. Kuenmer, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry

The antidepressant doxepin hydrochloride (DOX), the anticonvulsant gabapentin and the antipsychotic quetiapine hemifumarate (QUT) are widely used neurological drugs. They are frequently found in environmental compartments. Due to their high consumption and chemical stability, they are not fully eliminated by sewage treatment plants. Therefore, they are released to surface waters, like rivers, where they are exposed to sunlight irradiation. In this study the behavior of DOX, GAB and QUT was assessed undergoing simulated sunlight irradiation. Photolysis experiments in aqueous media were carried out in a 1000 mL batch immersion reactor using an xenon lamp. Primary elimination was analyzed by UV-C and the degree of mineralization by measuring non-purgeable organic carbon (NPOC) at specific time intervals, respectively. DOX was almost primary eliminated after two hours of irradiation. But the NPOC concentration still was only reduced by 5% after two hours and didn’t change until the end of the experiment (eight hours). The stability of the NPOC concentration alludes to the formation of several phototransformation products (PTPs). Single treatment of GAB and QUT didn’t lead to any change in parent compound or NPOC concentration. The mixture containing each API showed slower elimination kinetics for DOX due to competing effects. DOX was eliminated after four hours of light irradiation. The overall elimination of both APIs remained the same during the whole process. Mineralization was not observed. PTPs that were not fully eliminated by sunlight were not detected.

TH037 Ozone photolysis of trimipramine under different environmental conditions
A.O. Kondrakov, T. Glauner, Ignatiev, Lomonosov Moscow State University / Engler Bunte Institut Chair of Water Chemistry and Water Technology; A.N. Ignatiev, Lomonosov Moscow State University

The inclusion of sex and gender (s and g) is a significant challenge for environmental health studies. It is important to define what we mean by s and g, to demonstrate the added value of the data so generated when taken into account and to develop appropriate and relevant methods from an experimental point of view. But what about the environmental health studies conducted on animals in their natural environment? How ecotoxicologists define s and g in their studies? How to define the gender when working with wild animals in the laboratory? Are these two elements considered and named as such? How their influences are measured? What are the reasons that some make research on a specific topic to consider them and other not? What are the funding agencies requirements in this regard? What are the effects of taking or not s and g into account on the results? What impacts this consideration has on the methodological approaches used? How s and g are or aren’t integrated into the study requirements in relation to bioassays, biomarkers and biomarkers. We will try to answer those questions based on an analysis of the scientific literature of the effect of endocrine disruptors found in personal care products (phthalates, triclosan, bisphenol A and polyphenols) on shellfish and fish and hormonal and immune systems.

TH038 Ozone photolysis of trimipramine under different environmental conditions - biodegradation and LC-MSN characterization of the formed transformation products
N. Akhadeel, Leuphana Universität Lüneburg / Institute of Sustainable and Environmental Chemistry; O. Òlsön, Leuphana University Lüneburg; C. Kuenmer, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry

Trimipramine (TMP) is among widely used tricyclic antidepressant drugs throughout the world. It could cause toxic effects to humans and have a high risk of serious cardiovascular side effects; therefore it is important to study its potential environmental behavior. This research focuses on the effectiveness of ultraviolet (UV) radiation on the photodegradation of TMP, investigating the effect of different environmental variables (initial drug concentration, experimental temperature and solution pH) and different aqueous matrices (ultrapure, tap, surface
and wastewater) on the photodegradation process as well as on the formed photo-tranforming products (PTPs). The degree of mineralization was studied by following the time course of dissolved organic carbon (DOC) during the photolysis process. Liquid chromatography coupled to ion-trap mass spectrometry (LC-MS/MS) was employed to monitor and identify the formed PTPs. Biodegradation testing of TMP and samples after the photodegradation process was studied using biodegradation tests. The biodegradation rate was determined as COD (Chemical Oxygen Demand) and Manometric Respirometry test (MRT; OECD 301 F). Photodegradation kinetics followed first order reactions in all experiments except in wastewater matrix, which showed second order kinetics. The degradation was studied by following the DOC concentration. Comparison of the time courses of TMP photodegradation by HPLC showed that the corresponding DOC values indicate that TMP is transformed to new PTPs without complete mineralization. The previous phototransformation pathway of TMP in aqueous medium was by hydroxolation mainly. Finally, biodegradation tests revealed that neither TMP nor its PTPs were biodegradable under the studied conditions. Toxicity studies are required to be applied for the fate and occurrence of biocides are therefore crucial. To address this concern, sewage water and sludge from several sewage treatment plants located in Sweden were sampled and analysed. The concern for these substances has previously mainly been potential environmental health, therefore it is really important to evaluate the presence and the concentration of these pollutants in water bodies. In order to analyze these compounds, in this work a methodology with HPLC-MS-MS was developed and validated according to ICH Q2 R1 protocol. Two different kinds of C18 columns were applied to check which one would be better for the purpose of this work. The mobile phase was acetonitrile and acidified water pH 3.0, 50:50 in volume, 1.0 mL/min, injection volume 10 μL, temperature 40°C and wavelength 281 nm. The compounds were eluted. The bioavailability of these substances is of the utmost importance. Acknowledgement: This research has been financially supported by Ministry of Education, Science and Technological Development, Republic of Serbia (III46009).

TH042 Biocides in Swedish sewage treatment plants
M. Ostan, J. Fick, E. Björn, R.H. Lindberg, M. Tysklind, Umeå University / Department of Chemistry
Biocides are wide group of compounds that include substances with antimicrobial properties and substances that are used as preservatives in food and pharmaceuticals. The concern for these substances has previously mainly been potential ecotoxicological properties. However there are several indications that these substances might promote antibiotic resistance development in bacteria. To understand the fate and occurrence of biocides are therefore crucial. To address this concern, sewage water and sludge from several sewage treatment plants located in Sweden were sampled and analysed for the presence of biocides, antibiotics and pharmaceuticals with large structural variation under environmentally relevant conditions. Three different matrices, two of which contained DOM (reference materials received from IHSS) in environmentally relevant concentrations and one which did not, were placed under an ultraviolet (UV) light source (>300 nm) for up to 128 h. Samples taken at various time points were analyzed via an online SPE/LC-MS/MS system to establish the amount of photodegradation. Preliminary results indicate that the DOM matrices greatly affected photodegradation pathways considerably from a biodegradability point of view. Several substances rapidly released rapidly during lab-based UV-exposure did not do so. This presentation will report on half-lives of a large range of pharmaceuticals, which will be discussed in terms of their molecular structures and the IHSS provided quality data for the DOM substances.

TH043 HPLC-DAD Method Development and Validation for hormones in effluents
V. Bianchi, UFABC / CCNE; E. de Lima, Universidade Federal do ABC / Centro de Ciências naturais e Humanas
Estrogen hormones, such as estrone, estriol, 17α-ethinylestradiol and β-estradiol, are considered to be emergent pollutants, once they are not removed after usual sewage treatment. In addition, these substances are harmful for human and environmental health, therefore it is really important to evaluate the presence and the concentration of these pollutants in water bodies. In order to analyze these compounds, in this work a methodology with HPLC-DAD was developed and validated according to ICH Q2 R1 protocol. Two different kinds of C18 columns were applied to check which one would be better for the purpose of this work. The mobile phase was acetonitrile and acidified water pH 3.0, 50:50 in volume, 1.0 mL/min, injection volume 10 μL, temperature 40°C and wavelength 281 nm. The compounds were eluted. The biochemical and chemical oxygen demand (less than 3%) as well as good linearity (R² > 0.99). To check a possible interference, caffeine was added to a mixture of these hormones, and all of them could be efficiently separated. Therefore, the validated method is suitable for analysis of effluent samples in a practical way. Keywords: HPLC-DAD, hormones, effluents, effluent

TH044 Wastewater from meat processing industry-current quality issues
M. Vojnovic Milanovic; I. Mihaljovic, M. Milanovic, Medical Faculty, University of Novi Sad; N. Grgic Letic, Pharmacy, S. Pap, M. Brbaric, University of Novi Sad Faculty of Technical Sciences; Z. Njezic, Food Institute Novi Sad, University of Novi Sad; N. Milic, University of Novi Sad, Faculty of Medicine / Department of Pharmacy
Meat industry is one of the major sources of the organic pollution in the food sector. The emission of untreated or insufficiently treated wastewater is very common in developing countries and consequently has harmful effects to the environment. Biocides, antibiotics and pharmaceuticals are considered to be emergent pollutants once they are not removed after usual sewage treatment. In order to address this concern, sewage water and sludge from several sewage treatment plants located in Sweden were sampled and analysed for the presence of biocides, antibiotics and pharmaceuticals with large structural variation under environmentally relevant conditions. Three different matrices, two of which contained DOM (reference materials received from IHSS) in environmentally relevant concentrations and one which did not, were placed under an ultraviolet (UV) light source (>300 nm) for up to 128 h. Samples taken at various time points were analyzed via an online SPE/LC-MS/MS system to establish the amount of photodegradation. Preliminary results indicate that the DOM matrices greatly affected photodegradation pathways considerably from a biodegradability point of view. Several substances rapidly released rapidly during lab-based UV-exposure did not do so. This presentation will report on half-lives of a large range of pharmaceuticals, which will be discussed in terms of their molecular structures and the IHSS provided quality data for the DOM substances.

TH045 Evaluating the potential of constructed wetlands for attenuating pesticide losses from agricultural land to surface waters
A. Ramos; A.M. Ramos; V. Ioannidou; M.J. Wheater; R. Villa; I. Gayerou; B. Jefferson; Institute of Energy and Resource Technology, School of Energy, Environmental and Agricultural, Cranfield University, College Road, Cranfield, Bedfordshire, MK43 0AL, UK; a.m.ramos@cranfield.ac.uk; b.Jefferson@ Cranfield University, Cranfield, Bedfordshire, MK43 0AL, UK “Department of Geography, University of Leicester” University of Warwick
Some pesticides can periodically contaminate drinking water supplies at levels which create legal compliance challenges in Europe – particularly for substances which are difficult or expensive to remove using conventional water treatment technologies. A wide range of catchment management interventions have been suggested to reduce contaminant transfers to surface water abstraction points, including the construction of wetland systems on ditch and stream networks. Such wetlands could potentially reduce pesticide concentrations in water via a combination of degradation, sorption...
and hydrodynamic dispersion as well as providing other benefits such as wildlife habitat enhancement. Here, we evaluate the potential of three constructed wetlands to attenuate pesticide concentrations and loads in the surface waters of a commercial farm in central England. Water samples were collected at different locations using automatic water samplers and were monitored continuously using hydraulic structures (V-notch weirs and flumes). Samples were analysed using LC-MS-MS and GC-ECD. The analytes were extracted from 250 mL of water by solid phase extraction and determined by liquid chromatography triple quadrupole mass spectrometry using an electrospray ionization source in positive ionization mode. The method detection limits ranged from 0.01 to 1.54 ng L\(^{-1}\) and the recoveries from 57 to 127 %. The monitoring of these compounds in the Turia River Basin shows the presence of benzoyl glycine (metabolite of cocaine) in 68.2 % and 18.2 % at concentration up to 60.6 and 12.7 ng L\(^{-1}\) in 2012 and 2013 sampling campaigns, respectively. Another metabolite of cocaine is ecgonine methylester, and it was detected only in 2012 in 13.6 % at a concentration up to 108.5 ng L\(^{-1}\). Also, methadone was detected up to 6.3 ng L\(^{-1}\). The monitoring of these compounds in the Turia River Basin was found to be inappropriate in case of this study because of ambiguous test results. The microplate version of Ames test was found to be inappropriate in case of this study because of ambiguous test results.

**HI046** Distribution of polybrominated diphenyl ethers (PBDEs) in an aquatic ecosystem receiving effluents from a sewage treatment plant (STP) in Tongyeong, Korea.

H. Lee, Department of Marine Environmental Engineering; G. Kim, GYEONGSANG NATIONAL UNIVERSITY / Department of Marine Environmental Engineering

Ogun River, with the reservoir capacity of 690 million m\(^3\), is a significant river in the western states and empties into the Vistula river. The abattoir wastes, and at the same time as a source of drinking water for the residents along its bank. Consumers of the milk and meat products from the animals waiting to be slaughtered. The test was found to be inappropriate in case of this study because of ambiguous test results.

**HI047** HALOGENATED ORGANIC POLLUTANTS IN OGN RIVER AT KARA ABATTOIR, NEAR BERGER, LAGOS, NIGERIA

R. Alan, Department of University of Lagos; B. ALO, UNIVERSITY OF LAGOS; F. Ukoakonum, University of Lagos Nigeria / Chemistry

Ogun River, with the reservoir capacity of 690 million m\(^3\), is a significant river in the western states and empties into Lagos lagoon. Kara abattoir is one of the many abattoirs along the river's course. At Kara abattoir, Ogun River serves as a dump for all abattoir wastes, and at the same time as a source of drinking water for the animals waiting to be slaughtered. The river is equally used for domestic activities by the residents along its bank. Consumers of the milk and meat products from the abattoir could be at risk of food poisoning and health complications arising from exposure to dangerous organic pollutants. Other life forms in the total ecological community could also be at risk. Water and sediment samples were collected from Ogun River around Kara abattoir and analysed for Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) using gas chromatography with electron capture detector (GC/ECD). Concentrations of PCBs in water ranged from 0.007 to 0.030mg/L and from 0.015 to 0.037mg/L for the STP. These samples were then analyzed to test the presence of SPT effluent on distribution of PBDEs in the local aquatic ecosystem. The concentrations of total PBDEs (∑PBDEs) in seawater and surface sediments were found in the range 0.33-14 ng/g and 2.2-307 ng/g dry weight, respectively. Vertical concentration of sediment cores showed ∑PBDEs decreased exponentially with increasing depth. The bioconcentration factor (log BCF) exhibited a parabolic relationship with octanol-water partition coefficients (log Kow) values for PBDEs. This result suggests that Kow can be major factor governing bioaccumulation mechanisms of PBDE congeners.

**HI048** Ilicit drugs monitoring in the Turia River Basin

M. Andres Costa, Universitat de València / Environmental and Food Safety Research Group Department of Food Chemistry and Nutrition Faculty of Pharmacy University of Valencia; A.N. Fernandes, Institute of Chemistry; V. Andreu, CIDIE CSIC UV GY; Y. Pico, University of Valencia / Medicine Preventive

Until now, emerging contaminants have been found in many rivers such as Ebro, Jucar, Guadalquivir and Llobregat [1]. A recent study shows the presence of pharmaceuticals in the Turia River Basin [2]. The aim of this research was to track traditional and emerging illicit drugs in surface water in a typical Mediterranean river basin. Sampling campaign was carried out in 2 periods in 2012 and 2013. There were 22 sampling sites distributed along the river. A total of 45 metabolites of unchanged illicit drugs that belong to amphetamines, tryptamines, piperazines, pirlidinophenone, arylecyclohexylamine, alkaloid, opioids and cannabinoids groups were monitored. These compounds were extracted from 250 mL of water by solid phase extraction and determined by liquid chromatography triple quadrupole mass spectrometry using an electrospray ionization source in positive ionization mode. The method detection limits ranged from 0.01 to 1.54 ng L\(^{-1}\) and the recoveries from 57 to 127 %. The monitoring of these compounds in the Turia River Basin shows the presence of benzoyl glycine (metabolite of cocaine) in 68.2 % and 18.2 % at concentration up to 60.6 and 12.7 ng L\(^{-1}\) in 2012 and 2013 sampling campaigns, respectively. Another metabolite of cocaine is ecgonine methylester, and it was detected only in 2012 in 13.6 % at a concentration up to 108.5 ng L\(^{-1}\). Also, methadone was detected up to 6.3 ng L\(^{-1}\). The monitoring of these compounds in the Turia River Basin was found to be inappropriate in case of this study because of ambiguous test results.

**HI049** Monitoring of the genotoxicity and endocrine disrupting activity of Vistula river in the vicinity of Warsaw in Poland.

A. Zegadzka, Medical University of Warsaw / Department of Environmental Health Sciences; A. Skrzypczak, Environmental Health Sciences; J. Giebultowicz, Medical University of Warsaw / Department of Bioanalysis and Drugs Analysis; G. Nalecz-Jawicki, Medical University of Warsaw / Department of Hygiene

The bioconcentration factor (log BCF) exhibited a parabolic relationship with octanol-water partition coefficients (log Kow) values for PBDEs. This result suggests that Kow can be major factor governing bioaccumulation mechanisms of PBDE congeners.

**HI050** Pharmaceuticals and personal care products in the Turia River Basin

E. Carmona, University of Valencia / Environmental and Food Safety Research Group Department of Food Chemistry and Nutrition Faculty of Pharmacy University of Valencia; A.N. Fernandes, Institute of Chemistry; V. Andreu, CIDIE CSIC UV GY; Y. Pico, University of Valencia / Medicine Preventive

The term "emerging pollutants" stands for the substances that are released in the environment for which currently no regulations are established for their environmental monitoring. Their occurrence is reported worldwide in a range of environmental and marine waters. However, there are still few studies on their occurrence, levels and distribution in River Basins from Spain. This study is aimed at contributing information on the occurrence of pharmaceuticals and personal care products in the Turia River Basin, a typical Mediterranean River heavily affected by urban and industrial outflow. Selected compounds are related to more than 200 pharmaceuticals and personal care products and two illicit drugs. These substances were determined with an Agilent Technologies HPLC linked with a Triple Quadrupole LC/MS in positive and negative ion mode testing the application of NH\(_4\)F as salt in the mobile phase to improve the sensitivity. The optimal mobile phase was a gradient of 5mM Ammonium fluoride in water (mobile phase A) and 5mM Ammonium fluoride in methanol (mobile phase B) at a flow rate of 0.2mL/min with a gradient that starts with 30% of mobile phase B and increase until 95% at minute 12 and remains 13 minutes. The analytes were extracted from 250 mL of water by solid-phase extraction using Strata-X cartridges, eluted with methanol, evaporated and dissolved in 250 mL of methanol. This procedure provides acceptable recoveries.
TH051 Ecotoxicological risk of the seasonal bisphenol A concentration in the Danube along Novi Sad, Serbia
M. Milanovic, Medical Faculty, University of Novi Sad; L. RadeVIC, Faculty of Veterinary Medicine; M.M. Turk Sekulic, Department of Environmental Engineering and Occupational Safety and Health; M. Vojinovic Mlodorad; I. Mihajlovic; J. Sudij, University of Novi Sad / Faculty of Medicine; N. Grujic Letic, Pharmacy; N. Miloslevic, University of Novi Sad / Faculty of Medicine; N. Milic, University of Novi Sad, Faculty of Medicine / Department of Pharmacy.

Bisphenol A (BPA), as an organic compound extensively used in daily life products such as baby bottles, plastic containers and as a key component in medical diagnostic products, has been receiving the increasing attention due to the endocrine-modulating property to interfere with hormonal action and with the human cell functions mainly through the interaction with specific receptors. The presence of this synthetic estrogen in the environment is associated with a range of health disorders such as fertility problems, obesity, especially in children and adolescents, disrupted pancreatic b-cell and thyroid hormone function, cardiovascular disorders and increased carcinogenic risk. Despite its short half-life of about 0.5-5 days in the aquatic environment, the constant release into the surface water through direct discharge of municipal and industrial wastewater makes BPA a pseudo-persistent pollutant in the environment. According to all, the aim of the study was to monitor seasonal BPA concentrations and to assess the potential ecological risks during a one-year period. The surface water samples were collected in 12 amber glass bottles at eight representative locations along the Danube of the Novi Sad municipality, Serbia, during autumn, winter, spring and summer under the comparable meteorological conditions at all sites for each sample at the depth of 2 m. After the preconcentration step using Bond ElutPlexa type of cartridges, the seasonal concentrations of BPA were measured by the gas chromatography-mass spectrometry. Although, BPA was only detected in two samples in winter due to the weather conditions, high frequency of the detection was observed in autumn, spring and summer at significantly high concentration levels up to 692.6 ng/L. The environmental risk assessment was evaluated by comparing the 95th percentile of the measured environmental concentration for each sampling site and the predicted no-effect concentration (0.06 ng/L for BPA) in order to evaluate the BPA adverse effects on aquatic ecosystems. This calculated ratio ranged from 0.68 to 11.54 and the risk quotient was above 1 at seven locations. It can be concluded that the aquatic environment along the Danube of the point at the 1255 km, is not sufficiently protected from the adverse effects to the BPA exposure at the established water concentrations. Acknowledgement: This research has been financially supported by Ministry of Education, Science and Technological Development, Republic of Serbia (III46009) and NATO Science for Peace Project ESP.EAP.SFP 98/4087.

TH052 Effects of non-specific binding on the biotransformation rate of hydrophobic chemicals on an in vitro metabolism assay using rat liver S9 fraction
H. Lee; J. Kwon, Korea University / Division of Environmental Science and Ecological Engineering; B.I. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology.

Enzymatic transformation of highly hydrophobic compounds is amongst the most important factors modulating their persistence and bioaccumulation potential. It is well-known that hydrophobic chemicals strongly sorb to cellular matrices and this sorption affect their bioavailability. Physiologically-based pharmacokinetic models used for the derivation of whole-body bioaccumulation factor require the bioavailable fraction for enzymatic degradation. However, little is known about how enzyme kinetics is influenced by cellular binding or sorption. In order to elucidate effects of non-specific sorption, we measured apparent enzymatic kinetics in an in vitro assay using four hydrophobic polycyclic aromatic compounds (PAHs), phenanthrene, fluoranthene, pyrene and benzo[a]pyrene, as model compounds and S9 mixture isolated from rat liver as a model enzyme mixture. In addition, bovine serum albumin (BSA) was selected as non-metabolically active protein in order to investigate effects of protein binding. Rate constants were derived from measuring the depletion of parent PAHs over time. For benzo[a]pyrene, the rate constant was also calculated by measuring the formation of phase I metabolite to confirm the results from the parent depletion method. Apparent enzyme kinetics at various experimental conditions were examined and the results provide an important insight in the evaluation of bioaccumulation potential using PBPK modelling.

TH053 Pesticide residues in agricultural soil of Vojvodina Province, Serbia
S. Lazic; D. Sunjka; I. Milovcanovic, University of Novi Sad Institute for Food Technologies; M. Manoljovic, S. Vukovic, University of Novi Sad Faculty of Agriculture; P. Jovanov, Institute of Food Technology in Novi Sad.

Modern agriculture depends to a large extent on pesticides used for control of weeds, diseases and pests, in an effort to reduce or eliminate yield losses and preserve high product quality. Their prolonged use involves the risk of their retention and accumulation in the environment, so the analysis of these compounds has become an important part of modern farming practice. The aim of this study was to evaluate the presence of pesticide residues in soil under intensive agricultural production. The survey was conducted at 34 conventional farms in Vojvodina Province, the main agricultural area in the Republic of Serbia. In total, 60 soil samples from fields with different history of farming practices were analysed. Samples were collected in 3 cm layers, each 30 cm2 surface area from 0 to 30 cm soil depth and mixed. In total, 260 g of soil were formed and transferred to the laboratory. Laboratory samples of 100 g were air-dried, milled, sieved and analyzed. Extraction and clean-up procedure was carried out using modified QuECHERS method. For pesticides quantification gas chromatography separation with MS detection (Agilent Technologies GC-MS 7890 A Series, coupled with 5975C mass selective detector and HP 5 MS column) and high performance liquid chromatography with diode array detection (Agilent HPLC 1100 system and an Agilent Zorbax C18 column) were used. The performance characteristics of the method were established by validation procedures employing assays with standard solutions, sample blanks and spiked samples. Linearity, matrix effect, accuracy, precision, limits of detection and of quantification were determined. The suitability of this method was assessed successfully for the determination of commonly used pesticides. The obtained results indicated that all validation parameters were satisfactory and the method was applied on real soil samples. Results of the investigated soil samples showed the presence of pesticide residues from different classes - sulfonylureas, triazines and chloracetanilides herbicides, triazoles fungicides, as well as some insecticides. This research was carried out in the framework of the IPA project cross border cooperation Serbia-Croatia and project III43005 funded by Serbian Ministry of Education, Sciences and Technological Development.

TH054 Presence of the insecticide permethrin in indoor environment
S. Papaja, Secretaria municipal de Saúde de São Paulo / Supervisão Técnica de Saúde JardimPaula Vila Mariana; L.E. Nakagawa, Instituto Biológico / Laboratório de Toxicologia; C.M. Nascimento, A.R. Costa, R. Polatto, Instituto Biológico / Center for Research and Environmental Protection Development Pyrethroids insecticides are commonly used to control insects in residential homes in Brazil. Although pyrethroids offer lower toxicity to human and there are few reports of non-occupational poisoning, these insecticides may cause acute neurotoxic and immunotoxic effects, endocrine disruption and possible human carcinogens too. In addition, more frequent diseases such as allergies and respiratory problems are reported after application of these insecticides, especially in children and the elderly. Pyrethroids can remain for long time in indoor environment, since, unlike the ground, there is no microbial diversity to degrade the product. Furthermore, their chronic exposure is a major concern due to their potential to accumulate in the human body. The aim of this study was to evaluate the presence of the insecticide permethrin in a house test. Permethrin is of low to moderate persistence in the soil, with half-life between 30 and 38 days, being biodegraded by wide variety of edaphic microorganisms. A commercial product of the insecticide permethrin (emulsifiable concentrate, 38% imbc) was applied in a single dose on the floor of a house test. Soon after the application and after 14 and 28 days, the aluminum foils were collected and the insecticide was extracted using nhexane and quantified by high performance liquid chromatography. There was not degradation the insecticide permethrin 14 and 28 days after application. One hundred percent of the applied product remained in aluminum foil during this period. The results show prolonged persistence the product in an indoor environment where the people spend most of their time, especially children and the elderly. It is important understand the half-life of pyrethroids when applied indoor to reliable assessment of the actual risk of exposure. Support FAPESP 2013/17822-3

TH055 Seasonal Occurrence, Distribution and Ecological Risk Assessment of Multiple Classes of UV Filters in Marine Sediments
T.M. Mei Po Mirabelle, H. Leung, B.K. Kwan, K. Ng, City University of Hong Kong / Biology and Chemistry; N. Yamashita, National Institute of Advanced Industrial Science / Emtech; S. Taniyasu, AIST / Institute for Environmental Mngt Technologies; P. Lam, CSO / Emtech; P. Hong, CSO / Emtech; D. Sunjka; I. Milovanovic, University of Novi Sad / Faculty of Pharmacy; N. Milošević, University of Novi Sad / Faculty of Medicine; N. Milic, University of Novi Sad, Faculty of Medicine / Department of Pharmacy.

Ultraviolet (UV) radiation has become an important part of the monitoring program. The aim of this study was to characterize their sources and establish the real environmental occurrence of these potential contaminants. Further research will be devoted to characterize their sources and release pathways as well as to define and quantify processes that determine their transport and fate through the Turia River and to identify potential ecologic effects.
BMDP, BP-1, BP-3, BP-8, EHS, HMS, IAMC, OMC, OC and OD-PADA in sediment, (2) determine their occurrence and distribution in samples collected in Hong Kong and in Tokyo Bay, and (3) conduct a preliminary environmental risk assessment of these compounds in the marine environment. In Hong Kong, seven of the 11 target UV filters were detected in the samples, with median concentrations ranging from < LOD to 21 ng/g dw. Generally, the detection frequencies of UV filters were significantly higher than those in the dry season BMDP, BP-1, BP-8 and EHEM showed detection frequencies >50%. Composition profiles in Hong Kong and Tokyo Bay showed that BMDP, EHEM and ODPABA were the predominant compounds in sediment samples accounting for more than 60% of the total UV filter occurrence, while all of the benzoephonene derivatives accounted for < 35%, likely due to their relatively lower estimated soil organic carbon partition coefficients (log KOW) values (benzoephene-derivatives: 2.8-3.3; other detectable compounds: 4.4-6.8). Probabilistic ecological risk assessment showed that the likelihood of EHMC causing toxic effects on reproduction in snails was over 84% and 32% using toxicity thresholds based on data for two species, suggesting potential risks of UV filters to benthic organisms as well as possible wider effects on the marine food web.

The study involved monitoring of the compost quality and immune responses of earthworms and other invertebrates exposed to two types of biosolids (two types of anaerobically digested biosolids, one sourced from the UK and the other from Ireland; lime stabilised and thermally dried biosolids) and at three time intervals of 24, 48 and 360 hr from the time of application (all plots accounting for more than 60% of the respective total losses, but further buffering is possible further down the transfer continuum. Pharmaceutical testing of surface runoff is currently ongoing and may be decisive in determining whether biosolids may be safely applied to land. Keywords: Biosolids; soil runoff, contaminants, rainfall simulator

They also change the bioavailability of metals for plants, which may constitute a risk but also a great interest when employing these capacities in the treatment of the sewage sludge through vermicomposting process. This eco-technique is a non-expensive and rapid method of biodegradation of organic wastes, inter alia sewage sludge, commonly used in developing countries. The quality of the product can be assessed using agronomic parameters, while immune and defense parameters can be measured as well. The method used was: 1) to segregate worms coming from mixed colonies using DNA barcoding and fluorophores fingerprinting, 2) to assess the influence of pollutants on immune system (gene expression and immuneocyte sub-populations), 3) to assess the quality of the product by measuring physical and chemical parameters. Earthworms were segregated into three separated groups basing on DNA barcoding and selected fluorophores were used fluorometrically. earthworms allowing the distinction between morphologically similar coexisting composting earthworm species. Riboflavin, coelomocytes (amoebocytes/eocytes) composition and particular gene expression levels were selected as biomarkers of stress useful in biomonitoring of vermicomposting process. Applied technique has led to valorization of sewage sludge, by changing their physical and chemical properties and obtained product can be considered for future applications as fertilizer.

**Contaminants of Emerging Concern in Food Webs: Impacts on Non-Target Organisms (P)**

**TH058** Bio-physico-chemical behavior of photocrosslinked silicone acrylates S. OUALLY, J. LOUIS, P. GERMAIN, R. GOURDON, V. DESJARDIN, INSA de Lyon / Laboratory of Civil and Environmental Engineering Treated sewage sludge ("biosolids") may be applied to agricultural land as an organic fertiliser, as it offers an excellent source of nutrients and metals required for plant and crop growth. Due to the increasing awareness among the public and environmental health, the application of biosolids to land as a fertilizer has come under increased scrutiny in recent times. While there are many benefits accruing from the land application of biosolids, any nutrient recovery must be considered against possible adverse impacts associated with their reuse. The aim of this study was to quantify the loss of nutrients (phosphorus and nitrogen), metals (copper, nickel, lead, zinc, cadmium, aluminium, iron and manganese), faecal and total coliforms, and emerging pharmaceuticals (triclosan and triclocarban), under controlled, in-field conditions during and after successive rainfall simulation events. Four types of biosolids (two types of anaerobically digested biosolids, one sourced from the UK and the other from Ireland; lime stabilised and thermally dried biosolids) were surface applied to replicated (at n=5) hydraulically isolated micro-plots, each 0.9 m long x 0.4 m wide. Prior to application, the biosolids and plots were fully characterised for nutrient, metal, microbiological and pharmaceutical parameters of interest. Rainfall with an intensity of 11 mm h⁻¹ was applied to each plot using a portable multi-drop rainfall simulator, over three successive 30 min rainfall events at various times after the application. Micro-plots were covered by rain-out shelters. Runoff water samples were collected and fully characterised. The flow-weighted mean concentrations (FWMC) measured in the surface runoff indicated that nutrient losses, while high, were similar to other organic wastes, metal concentrations were well below their respective drinking water limits, and faecal coliforms did not persist as long, and were lower than untreated cattle slurry. The FWMC measured in this study represented a 'worst case' scenario for potential losses, but further buffering is possible further down the transfer continuum. Pharmaceutical testing of surface runoff is currently ongoing and may be decisive in determining whether biosolids may be safely applied to land. Keywords: Biosolids; soil runoff, contaminants, rainfall simulator

**TH057** Assessment of the vermicomposting process applied to sewage sludge by monitoring of the compost quality and immune responses of earthworms A.B. Rorat, Teagasc Environment Research Centre / Environmental Research; M.G. Healy, NUI Galway / Civil Engineering; D.P. Peyton, Teagasc Environment Research Centre / Environmental Research; M. Cantwell, Teagasc the Irish Agriculture and Food Development Authority / Food Research Centre Ashtown Co Dublin; G.T. Fleming, NUI Galway / Microbiology Co Galway; J. Grant, Teagasc the Irish Agriculture and Food Development Authority / Food Research Centre Ashtown Co Dublin; D. Wall, Teagasc the Irish Agriculture and Food Development Authority / Environmental Research Centre Johnstown Castle Co Wexford; O. Fenton, Teagasc the Irish Agriculture and Food Development Authority / Environment Research Centre Johnstown Castle Co Wexford

The aim of this work was firstly to synthesize photocrosslinked silicone acrylate elastomers, then to release the biodegradation of polymer and PI over time under aerobic and anaerobic conditions. Laboratory experiments were conducted in order to simulate the end of life conditions for silicone acrylates, whether in wastewater treatment plants or in landfills. Both physico-chemical and molecular biological techniques (molecular fingerprint TTGE and qPCR) were used to study the effect of elastomers on bacterial and fungal communities under the tested conditions. The results show that the PI is a very mobile compound, almost all of the amount used to synthesize the elastomers was recovered after 10 days of experiments. For a broad variety of applications, such as photocurable coatings, printing inks and adhesives, which all require the use of a photoinitiator (PI). As an UV PI, Darocur 1173 (2-Hydroxy-2-methyl-1-phenyl-propan-1-one) is widely available and well investigated. However, the environmental issue is the possible dispersion of chemicals both during the use and at the end of the product. Although the PI represents only a minor part of the formulation, this emission process is closely related to the migration of these molecules. In fact, the most severe requirements regarding migration properties are imposed only on UV curable printing inks for food packaging applications and for pharmaceutical products. Regarding the end of life, the behavior of silicone in wastewater treatment plants and landfills has been poorly studied and their possible environmental impact is not known. Furthermore, to the knowledge no studies have been published on the behavior and fate of photocrosslinked silicone acrylates. Thus, the aim of this work was to synthesize photocrosslinked silicone acrylate elastomers, and then to assess the release of PI by leaching test. Finally to assess the biodegradation process of elastomers and PI over time under aerobic and anaerobic conditions. Laboratory experiments were conducted in order to simulate the end of life conditions for silicone acrylates, whether in wastewater treatment plants or in landfills. Both physico-chemical and molecular biological techniques (molecular fingerprint TTGE and qPCR) were used to study the effect of elastomers on bacterial and fungal communities under the tested conditions. The results show that the PI is a very mobile compound, almost all of the amount used to synthesize the elastomers was recovered after 10 days of experiments. For a broad variety of applications, such as photocurable coatings, printing inks and adhesives, which all require the use of a photoinitiator (PI). As an UV PI, Darocur 1173 (2-Hydroxy-2-methyl-1-phenyl-propan-1-one) is widely available and well investigated. However, the environmental issue is the possible dispersion of chemicals both during the use and at the end of the product. Although the PI represents only a minor part of the formulation, this emission process is closely related to the migration of these molecules. In fact, the most severe requirements regarding migration properties are imposed only on UV curable printing inks for food packaging applications and for pharmaceutical products.

**TH055** Accumulation and depuration of per- and polyfluorinated compounds from bottom sediment by a freshwater amphipod (Gammarus spp.): assessment of exposure routes, accumulation and depuration kinetics D. Benne, I. Iovita / Water MALY Ecotoxicology Laboratory; M.P. Babut, Iovita / Water; G. Munoz, LPTC EPOC 5805 CNRS / UMR EPOC LPTC; P. Labadie, H. Enders, H. Fenton, Teagasc the Irish Agriculture and Food Development Authority / Environment Research Centre Johnstown Castle Co Wexford; M.G. Healy, NUI Galway / Civil Engineering; M. Danaher, H. Cantwell, Teagasc the Irish Agriculture and Food Development Authority / Food Research Centre Ashtown Co Dublin; G.T. Fleming, NUI Galway / Microbiology Co Galway; J. Grant, Teagasc the Irish Agriculture and Food Development Authority / Food Research Centre Ashtown Co Dublin; D. Wall, Teagasc the Irish Agriculture and Food Development Authority / Environmental Research Centre Johnstown Castle Co Wexford; O. Fenton, Teagasc the Irish Agriculture and Food Development Authority / Environment Research Centre Johnstown Castle Co Wexford

The amphipod Gammarus spp. were collected above polluted areas and many studies indicated their ability to accumulate pollutants, such as Trace Metals (MTes).

**Keywords:** Biosolids; soil runoff, contaminants, rainfall simulator
Bioaccumulation of perfluoralkyl compounds by benthic invertebrates and fish downstream of an industrial platform in the Rhone River (France) M. P. Babit, Isetra / Water; P. Labadie, University of Bordeaux / UMR EPOC Equipe LPTIC, D. Bertin, Isetra / Water MALY Ecotoxicology Laboratory; G. Munoz, LPTCEP 5805 CNRS / UMR EPOC LPTIC; H. Budzinski, University of Bordeaux / UMR EPOC Equipe LPTIC

Following a screening study at the Rhone watershed scale addressing fish contamination in 2009–2010, a research focusing on the trophic networks of three cyprinid fish species (Barbus busbus, Gobio gobio, Batillus rutulus) is being implemented. It consists in measuring fish biomarkers and levels of lipid peroxidation in mussel’s digestive gland. Toluene and tetrachloroethylene were the compounds detected in higher concentrations in fish and levels of lipid peroxidation in mussel’s digestive gland. Possible relations of biological data with contaminants’ levels in water, sediments and mussel tissues were also characterized, whenever possible. The biota to sediment accumulation factors (BSAf) for C7 to C11 perfluorinated carboxylic derivatives and C8 perfluorinated sulfonates were determined on the basis of invertebrates contamination data. Fish contamination was influenced by PFASs characteristics, fish feeding behavior, and might be also influenced by their physiology. In the case of barbel (B. barbus) for some PFASs young fish were significantly more contaminated than adult ones, while for other compounds adults were more contaminated, though not significantly except for PFTEda (perfluorotetracanecidoic acid). Biomagnification factors (BMsfs) were determined for two pairs of predator–prey, namely gudgeon (G. gobio) – chironomids, and barbel – gaimardi. PFNA (perfluorononanoic acid) and PFDoA (perfluorododecanoic acid) were also characterized, whenever possible. The increased awareness and concern focused on understanding their environmental fate and toxicological effects. The aim of this study was to increase knowledge on contamination by HNS in NW Portuguese estuaries of Minho and Douro rivers. Samples were collected in the spring of 2014. Twenty five HNS, including those in water, sediments and biota contamination in NW Portuguese estuaries of Minho and Douro rivers. For making an ecological risk assessment of these contaminants. Twenty five HNS were screened in water, sediments and mussels (Mytilus sp.) collected along the NW Portuguese coast. Samples were collected in spring of 2014 in six marine beaches. HNS were measured using solid-phase micro-extraction and gas chromatography with ion trap mass spectrometric detection. Additionally, biomarkers of exposure to contaminants (gluthione-S-transferase, catalase, glutathione reductase, glutathione peroxidase and superoxide dismutase activities; and levels of lipid peroxidation and oxidation of proteins) were evaluated in mussel’s digestive gland. Toluene and tetrachloroethylene were the compounds detected in higher concentrations in water and sediment samples, respectively. Significant differences in biomarker levels were observed between the sampling sites. Compared to fresh water, there was a significant decrease in the activity of antioxidant enzymes and in the levels of lipid peroxidation in mussel’s digestive gland. Possible relations of biological data with contaminants’ levels in water, sediment and mussel tissues will provide important information regarding the baseline situation of the study area and potential biological effects, and will be further discussed in this communication. Acknowledgments: This work was funded by the Project ECORISK (reference NORTE-07-0124FEDER-000055) within the SR&TD Integrated Program MARVALOR - Building research and innovation capacity for improved management and valorization of marine resources, supported by the Programa Operacional Regional do Norte (ON.2 – O Novo Norte) and by the European Regional Development Fund.
Often estuarine organisms suffer the simultaneous impact of man–made chemicals and natural stressors. Although, this usually results in occurrence of synergistic and/or antagonistic interactions, limited information is yet available on such combined effects have been described. This study, thus, investigated the combined effects of seastar (SERT) and to cellular processes influenced by salinity stress, and provided a link between physiological changes and possible ecological consequences. These biomarkers are widely recognized as useful early-warning tools to evaluate environmental contamination. The effects of two environmentally realistic concentrations of SERT were assessed at two salinity levels: brackish water (BW) and seawater (SW). Significant accumulation of SERT was only found in crabs exposed in SW. Synergism and antagonism were identified for biomarkers of cholinergic neurotransmission, antioxidant defences, and oxidative damage. The biomarkers tested appear to be more sensitive than common regulatory endpoints like pleural, gill index measured in daphnids. Different interactions (synergism and antagonism) between salinity and SERT were found. Differences were also observed for interactions involving low and high SERT concentrations. The results support the urgent need for multiple stressor evaluations, involving emerging contaminants of concern and natural stressors associated to global climate changes, to improve ecological risk assessment for these chemicals. This work was funded by the EU-FEDER ECOISMISD reference NORTE-07-0124-FEDER-000055) within the SR&TD Integrated Program MARVALOR - Building research and innovation capacity for improved management and valorisation of marine resources, supported by the Programa Operacional Regional do Norte (ON.2 – O Novo Norte) and by the European Regional Development Fund; and by the Portuguese Foundation for Science and Technology (FCT) through project PEst-C/MAR/LA0015/2013.

TH065

Effluent from an NSAID-manufacturing plant in Mexico induces genotoxicity on Cyprinus carpio

L.M. Gómez-Oliván, Universidad Autónoma del Estado de México, Fac. de / Facultad de Química; Sanborn Reyes, O. Dublán-García, Universidad Autónoma del Estado de México / Facultad de Química; M. Galar-Martínez, Laboratory of Aquatic Toxicology Department of Pharmacy; S. Garcia-Medina, National School of Biological Sciences, IPN / Laboratory of Aquatic Toxicology, Department of Pharmacy.; H. Islas-Flores, Instituto Politecnico Nacional / Escuela Nacional de Ciencias Biológicas

The effluent from the production of a non-steroidal anti-inflammatory drug (NSAID) generates large quantities of wastewater varying in characteristics and concentration as a function of the manufacturing process used and the year season. These effluents originate mainly as a result of machinery and, in addition to residues of the pharmaceutical products manufactured (as nonsteroidal anti-inflammatory), also contain other kinds of compounds used in the cleaning process, including solvents (ethanol) and detergents such as sodium dodecylbenzenesulfonate (SDBS). There is some evidence that these products generate reactive oxygen species, causing lipid, protein, and deoxyribonucleic acid damages, by the mechanism of oxidative stress. The aim of this study is to evaluate the genotoxicity induced by an effluent from an NSAID-manufacturing plant in Mexico on Cyprinus carpio. Initially, industrial effluent was sampled and obtained and acclimation of Cyprinus carpio was conducted. The median lethal concentration (LC50) and subsequently the lowest observed adverse effect level (LOAEL) were determined. Sublethal study of the organisms (genotoxicity test) was realized using LOAEL. Exposure times were 12, 24, 48, 72 and 96 h. The damage was evaluated using the comet (Tice et al., 2000) and micronucleus assay (Albertini et al., 2000; Lindberg et al., 2007). The LC50 obtained was of 1.137 %; Results show a significant difference with the control group (p < 0.05) after 24 h exposure in the comet and micronucleus assay. In conclusion this industrial effluent induces genotoxicity on Cyprinus carpio.

TH066

Evaluation of toxicity induced by hospital wastewater effluent on Cyprinus carpio

L.M. Gómez-Oliván, Universidad Autónoma del Estado de México, Fac. de / Facultad de Química; Neri-Cruz, O. Dublán-García, Universidad Autónoma del Estado de México / Facultad de Química; M. Galar-Martínez, Laboratory of Aquatic Toxicology Department of Pharmacy; S. Garcia-Medina, National School of Biological Sciences, IPN / Laboratory of Aquatic Toxicology, Department of Pharmacy.; H. Islas-Flores, Instituto Politecnico Nacional / Escuela Nacional de Ciencias Biológicas

The very wide range of activities performed by hospitals (care, diagnostics, hygiene, maintenance, research) lead them to use a great variety of potentially ecotoxic substances, such as surfactants, disinfectants, and pharmaceuticals. The contact of these pollutants with aquatic ecosystems leads to a risk directly related to the existence of hazardous substances that could have potential negative effects on biological balance of natural environments. This study aimed to determine oxidative stress induced on the common carp Cyprinus carpio by effluent from a hospital in Mexico. The median lethal concentration (LC50) and subsequently the lowest observed adverse effect level (LOAEL) were determined. Carp were exposed to different concentrations and treatment was monitored for different exposure periods (24, 48, 72 and 96 h) and the following biomarkers were evaluated: liver, brain, kidney, blood, and bile, hydroperoxide content (HPC), malondialdehyde content (MDA), protein carbonyl content (PCC), and the activity of the superoxide enzymes superoxide dismutase (SOD) and catalase (CAT). Statistically significant increases with respect to the control group (p < 0.05) were observed in HPC, L-PX and PCC particularly in gill, liver, and brain. Significant differences in the effluent activity in liver and brain also increased with respect to the control group. This particular hospital effluent is therefore concluded to induce oxidative stress on C. carpio, this damage being most evident in gill, liver and brain.

TH067

A survey of benthic sediment contaminants in reaches of the Columbia River Estuary based on channel sedimentation characteristics


While previous studies have documented contaminants in fish, sediments, water, and wildlife, few specifics are known about the spatial distribution of contaminants in the Columbia River Estuary (CRE). Our study goal was to characterize sediment contaminant detections and concentrations in reaches of the CRE that were concurrently being sampled to assess contaminants in water, invertebrates, fish, and osprey (Pandion haliatus) eggs. Our objectives were to develop a survey design and sediment transport model to predict sedimentation characteristics of three 16 km river reaches in the CRE. We then compartmentalized the modeled change in bed mass after a two-week simulation to define sampling strata (strata) with depositional, stable, or erosional conditions. We collected and analyzed bottom sediments to assess whether substrate composition, organic matter composition, and contaminant concentrations and detections varied among strata within and between reaches. We observed differences in grain size fractions between strata within and between reaches. We found that the fine sediment fraction was positively correlated with TOC. Contaminant concentrations were statistically different between depositional vs. erosional strata for the industrial compounds, personal care products and polycyclic aromatic hydrocarbons class (Indus-PCP-PAH). We also observed significant differences between strata in the number of detections of Indus-PCP-PAH ( depositional vs. erosional; stable vs. erosional) and for the flame retardants, polychlorinated biphenyls, and pesticides class ( depositional vs. erosional, depositional vs. stable). When we estimated mean contaminant concentrations by reach, we observed higher contaminant concentrations in the furthest downstream reach with a decreasing trend in the two upstream reaches. Contaminant survey designs that account for sedimentation characteristics could increase the probability that sampling is allocated to areas likely to be contaminated.
males showed the following distribution: gills (3.8) > hepatopancreas (4.3) > muscle (1.5). OCPs levels in males from RH decrease with the increment of the carapace width with the major difference in hepatopancreas of organisms with >30 mm (4.8) and < 25 mm (8.3), showing a dilution effect of pollutants. In all samples analyzed, endosulfans represented up to 70% of OCPs, with a ρ-b-isomers ratio >1. This pattern indicates the technical mixture application for agricultural proposes, until their recently ban in Argentina. Enrichment in endosulfan sulfate was found in hepatopancreas in both species, suggesting the metabolism of the parent isomers in these organisms. The pollutants profile observed in crab tissues were similar to those found in water and BS, showing that C. angulatus is a suitable biomonitor for organic pollutants. This study revealed that land use in surrounding areas represents current sources of OCPs and PCBs to Bahía Blanca estuary. Consequently, future long-term monitoring programs in coastal areas are necessary in order to reduce the impact from agriculture and industrial activities on non-target communities.

TH069
Formation and occurrence of the pyrethroid degradation product 3-PBA in urban waterways
J. Richards, University of California Riverside / Environmental Science; W. Jiang, California Environmental Protection Agency / Department of Pesticide Regulation; J. Gan, University of California, Riverside / Department of Environmental Science
Pesticides are used in urban environments to control the populations of undesirable organisms such as ants and termites. The most intensive urban pesticide use occurs in areas like Southern California with a large human population density and a climate that allows pest species to be active almost year-round. For example, over 3.5 million pounds of pesticide active ingredients were applied to urban environments in California during 2012. In California, urban insecticide use is dominated by pyrethroids. While the occurrence of pyrethroid parent compounds has been widely documented in urban watersheds, the occurrence and formation of their degradation products has largely been ignored, even though their common metabolite 3-phenoxybenzoic acid (3-PBA) is a known endocrine disrupting compound. In this study, the formation of 3PBA was investigated by spiking concrete slabs with three different formulations containing permethrin and the concentration of 3-PBA in runoff was determined. The occurrence of 3PBA in urban waters was also determined by collecting runoff samples from 6 locations in southern California, as well as in urban streams. Rapid transformation of concrete applied permethrin to 3-PBA was observed with 3-PBA levels in runoff exceeding 100 μg L\(^{-1}\) even 3 months after the initial application. Similarly, the occurrence of 3-PBA in urbanized waterways was found to be widespread. These findings suggest that concrete surfaces may play an important role in the transformation of pyrethric compounds, and that the inclusion of pesticide degradation products, such as 3-PBA, should be considered in monitoring and risk assessment in the future.

TH070
Pyrethroid-Induced Oxidative Stress in a Model Social Insect
L. Jensen, Entomology; D. Swale, Vanderbilt Medical Center / Anesthesiology; M.J. Lydý, Southern Illinois University-Carbondale / Center for Fisheries Aquaculture and Aquatic Sciences; T.D. Anderson, Virginia Tech / Entomology
The honey bee (Apis mellifera L.) is the most widely managed crop pollinator in the United States providing the agricultural industry with the economic viability needed to satisfy the food and fiber needs of society. The annual losses of managed bee colonies are an enormous financial burden on the apiculture and agriculture industries. A perceived problem for the reduce number of bee colonies is the excessive use of agricultural and urban insecticides. Pyrethroid insecticides elicit oxidative stress via the unregulated accumulation of the reactive oxygen species in model insects. In turn, these reactive oxygen species can reduce immunocompetence, reproductive quality, and longevity in the insect. There are several gaps in our knowledge with respect to the effects of pyrethroids on insecticide-mediated oxidative stress in bees. The ATP-sensitive potassium channels (K\(_{ATP}\)) are a member of the inward-rectifying potassium channel (K\(_{IR}\)) family that provide cellular protection against oxidative stress. These K\(_{ATP}\) channels function as cellular osmoregulators to restore membrane potentials and maintain intracellular oxygen species at acceptable levels during oxidative stress. Here, we will present the physiological mechanisms responsible for regulating oxidative-stress in bees following pyrethroid insecticide exposures. The K\(_{ATP}\)-mediated regulation of oxidative stress in pyrethroid-treated and -untreated bee genotypes will be reported using a classical pharmacological approach with K\(_{ATP}\) channel activating and deactivating compounds. We will discuss the use of these drugs to advance understanding of the effects of insecticide exposures on bees, and the mechanisms that protect bees against these stressors in an effort to improve the health and productivity of bee colonies.

TH071
Are neonicotinoids a threat for leaf litter breakdown? A laboratory approach with leaf shredding invertebrates
D. Engelt, Institute for Environmental Sciences / Institute for Environmental Sciences; M. Link, Universität Koblenz-Landau / Institute for Environmental Sciences; J.P. Zumbro, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundersch, Department of Agricultural Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment
Neonicotinoids are registered in over 120 countries for use against herbivorous insects in agriculture, horticulture, forestry, and tree nursery. Due to their extensive application and chemical properties, neonicotinoids are susceptible to be transported into surface waters via runoff pathways. There they might cause a threat to target species and associated ecosystem functions, such as the breakdown of leaf litter. This particular ecosystem function is critical in providing energy for local as well as downstream communities. To assess for potential risks of three frequently used neonicotinoid insecticides (imidacloprid, thiacloprid and acetamiprid) in this process, Gammauros fassorum (Amphipoda) and Cheopepteryx villosa (Thrioptera) were chosen as test species. These organisms were exposed to a range of neonicotinoids concentrations using their leaf consumption as endpoint. All experiments revealed a concentration-dependent decrease in the response variable, while the amphipod G. fassorum was 3 to 9 times more sensitive towards neonicotinoid exposure than the insect larvae C. villosa. Furthermore, when comparing the effective concentrations causing 20% reduction in feeding (EC\(_{20}\)) expressed as μg/mL), thiacloprid was irrespective of the species investigated – the most toxic neonicotinoid. Moreover, for Gammauros, all three EC\(_{20}\) (1.7 to 3.6 μg/L) were in the range of field concentrations. Therefore, the present study indicates a risk for the leaf litter breakdown mediated by invertebrates, which might restrict the energy availability (in the form of feces) e.g. for collectors. Such implications may consequently reduce the prey availability for aquatic (e.g. fish) and – following the emergence of insects – terrestrial predators (e.g. spiders, birds, bats).

TH072
Dietary exposure may increase the toxicity of roxithromycin in zebrafish (Danio rerio)
J. Soh, Seoul National University; S. Lee, Seoul National University / School of Public Health; H. Shin, Seoul Nation University; C. Kim, School of Public Health, Seoul National University; D. Jung, Seoul National School / Institute of Health and Environment; K. Choi, Seoul National University / School of Public Health
Among various chemical exposure pathways, especially dietary exposure is one of the most important exposure pathways of bioaccumulative chemicals to aquatic organisms. However, studies involving dietary exposure have rarely been conducted for pharmaceuticals. Roxithromycin (RTM) is a widely detected antibiotic in aquatic environment, and is suspected for bioaccumulation. We conducted a food web exposure study on RTM in zebrafish (Danio rerio) employing Artemia nauplii as a feed. Zebrafish were exposed via water only (Group A) or via both water and food (Group B). Water concentrations were determined at 0.05, 0.5, or 5 mg/L following preliminary range finding tests. Dietary exposure was conducted using Artemia that were exposed to RTM at the same concentration for 24 hours after hatch. During exposure period, zebrafish were sampled on days 7 and 14, and were measured for molecular level markers of exposure to RTM. Transcription of sodl gene in liver tissue of zebrafish in Group B showed positive trends both on day 7 and day 14. However, there were no significant differences in the transcription of sodl gene in Group A. Transcription of cypla was up-regulated in Group B on day 14, also the transcription of cyp3a in Group B was significantly increased at 5mg/L. However there were no significant change in Group A. The transcription of cyp3a4 gene in liver tissue of zebrafish was up-regulated on day 7 in Group A. In brain, positive trends of ache gene transcription was found in Group B on day 7 but the difference disappeared on day 14. Overall, our results show that RTM can be transferred to fish through dietary exposure, and increase the adverse effects in fish. Food web exposure or secondary poisoning of pharmaceuticals should be considered in risk assessment of pharmaceuticals in aquatic environment.

Emerging Contaminants in the Marine Environment: Presence, Effects, Regulation (P)

TH073
Mapping of emerging and legacy polyfluorinated substances in sediment and in-dwelling organisms and their diet: validation of a new method for non-target monitoring and partitioning in a macro-tidal highly turbid estuary (Gironde, SW France)
G. Munoz, LPTCEPOC 5805 CNRS / UMR EPOC LPTC; H. Budzinski, P. Labadie, University of Bordeaux / UMR EPOC Equipe LPTC
Polyfluorinated substances (PFASs) are man-made chemicals that have been in use since the 1950s due to their excellent tensioactive properties. However, several PFASs have been reported to exhibit persistent, bioaccumulative and toxic properties; hence the interest in the analysis of these compounds in all environmental media. However, data for estuarine environments are scarce. Estuaries are complex systems, located at the land/sea interface, and are therefore of primary concern as regards the transfer of micropollutants to marine and coastal ecosystems. In this context, the 2014 HALOGIR campaign aimed at better understanding the ecodynamics of halogenated contaminants of emerging concern, including PFASs, in a macro-tidal highly turbid estuary. Located in the South West of France, the Gironde estuary was selected as our study site because of its ecological value, being home to a large number of diadromous and anadromous
fish, and the last known spawning site of the European sturgeon. The purpose of the present work was (i) to determine the PFAS contamination levels in the Gironde estuary, targeting both legacy and emerging compounds, (ii) to investigate the physico-chemical factors controlling PFAS levels in the water column (dissolved phase and suspended particulate matter (SPM)), and (iii) to extend and deepen knowledge of the physico-chemical factors controlling PFAS sedimentary accumulation. The sampling strategy consisted in a South-to-North transect, following a salinity gradient from the confluence of the Garonne and Dordogne rivers to the mouth of the estuary at Pointe-du-Verdon. A fine sediment deposition area, located approximately 25 to 50 km West to the mouth of the estuary, was also included in our survey to perform preliminary assessment of the PFAS transfer from the Gironde estuary to the Bay of Biscay continental shelf. The HALOCLIR campaign took place September 2014 and targeted 23 sampling locations. Subsurface and bottom water samples, as well as bottom sediment samples, were collected at each site. At the same time, 13 additional sediment samples were collected at low tide across tidal mudflats, on both banks of the estuary. Preliminary results indicate that PFASs were ubiquitous in the dissolved phase, 16/26 investigated PFASs being reported at sub ng.L⁻¹ levels. Following PFAS analysis of SPM and sediment samples, the influence of environmental variables on PFAS levels, patterns and partitioning will also be evaluated.

**TH074 Occurrence and concentrations of benzotriazole UV stabilizers in sediments from industrialized coastal regions, South Korea**

M. Rani, Korea Institute of Ocean Science and Technology; S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; M. Jang, Korea Institute of Ocean Science and Technology / oil and POPs research group; OPRG; G. Han, Korea Ocean Research and Development Institute / Oil and POPs research group; Y. Song, Korea Institute of Ocean Science and Technology; W. Shim, Korea Institute of Ocean Science and Technology / Oil and POPs research group; Y. Jeon, Korea Institute of Ocean Science and Technology / Oil and POPs research group; Y. Song, Korea Institute of Ocean Science and Technology / Oil and POPs research group; Y. Jeon, Korea Institute of Ocean Science and Technology / Oil and POPs research group; Y. Jeon, Korea Institute of Ocean Science and Technology / Oil and POPs research group; Y. Jeon, Korea Institute of Ocean Science and Technology / Oil and POPs research group.

Benzotriazole UV stabilizers (BUVSs) are a class of plastic additives that are used in polypropylene and ABS (Acrylonitril, Butadiene and Styrene) copolymer products, which in turn are employed in building, automobile and consumer materials. Among several BUVs, 2-(3-[(butyl-2-hydroxy-5-methylphenyl]-5-chlorobenzotriazole (UV-326), 2,4-di-t-butyl-6-(3,5-chloro-2H-benzotriazole-2-yl) phenol (UV-327), 2-(2H-benzotriazole-2-yl)-4,6-di-t-pentylphenol (UV-328) are commonly used and toxic to alterations in sex hormones, developmental effects etc. The (2H-benzotriazole-2-yl)-4,6-bis(1,1-dimethylethyl)-phenol (UV-320) is prohibited from production, usage and import, because of its potential for bioaccumulations and toxicity to liver and gonads of aquatic animals. However, environmental data on these compounds are still very limited. Therefore, level and distribution of these BUVs were investigated in sediments (n=84) from two highly industrialized coastal regions in South Korea i.e. Ulsan (n=47) and Busan (n=37) with different characteristics (i.e., population and potential source in the catchment). Their frequent detection ranged from n.d.-174 (UV-320), n.d.-168 (UV-326), n.d.-168 ng/g dw (UV-328) indicated widespread use. The overall average concentration was highest for UV-320 (19.2±34 ng/g dw) followed by UV-328 (14.6±26 ng/g dw), UV-327 (8.9±19 ng/g dw) and UV-326 (6.9±22 ng/g dw) pointed out concern for possibly affecting the vital activities of the marine animals via BUVs. The concentration of BUVSs was higher in Ulsan than that of Busan. Industries followed by harbor and river discharge can be considered as BUVs input sources to marine environment. At both regions, the BUVS concentrations showed a negative gradient from land (source) toward offshore region, indicating land based activities are the main source of BUVSs to the marine environment. Widespread detection of these contaminants of emerging concern in marine sediments suggests that these chemicals have the potential to migrate out of consumer products and enter the outdoor environment.

**TH075 FABRIC PHASE SORPTIVE EXTRACTION (FPSE) FOLLOWED BY ULHPLC FOR THE ANALYSIS OF UV STABILIZERS IN AQUEOUS SAMPLES FROM GRAN CANARIA ISLAND (SPAIN)**

S. Montesdeoca, Departamento de Sedimentología, Universidad de Las Palmas de Gran Canaria / Chemistry, Z. Sosa-Ferreira, Universidad de Las Palmas de Gran Canaria / Departamento de Química; A. Kabir, Universidad de Las Palmas de Gran Canaria; K.G. Furtón, Florida International University; J. Santana-Rodriguez, Universidad de Las Palmas de Gran Canaria / Departamento de Química

Fabric phase sorptive extraction (FPSE) is a novel sample preparation technique developed by Kabir and Furtón [1]. FPSE is a highly sensitive, fast, efficient and solvent minimized sample preparation approach [2] that integrates the advantages of sol-gel derived hybrid inorganic-organic sorbents and the flexible, permeable and rich surface chemistry of cellulose fabric. FPSE media contains approximately 400 times higher sorbent loading than SPME fibers, providing better extraction efficiencies. Moreover it is a cheap device that can be reused and do not suffer from coating damage, often encountered in SPME fibers or stir bars. Unlike SPME or other microextraction techniques, FPSE mimics solid phase extraction (SPE) by allowing permeation of aqueous sample matrix through its extraction media, resulting in a near-exhaustive extraction (with very high absolute recovery of analytes) in a short period of time. Benzotriazole UV filters are a group of compounds added not only in sunscreen but also in variety of personal care products, which present negative effects over aquatic systems. They are mutagenic in bacterial systems and toxic to plants and can exert adverse effects on the fecundity and reproduction of fish [3]. We optimized the extraction of seven benzotriazole UV filters evaluating three different FPSE sorbent chemistries (polyethylene glycol, polytetrahydrofuran and poly(dimethylsiloxane)). All tested parameters were tested, such as extraction time, desorption solvent and ionic strength. The extraction procedure was followed by ultra-high pressure liquid chromatography with mass spectrometry detection. Once optimized the sample preparation and analysis procedure, the analytical parameters were evaluated and it was applied to determine the presence of seven benzotriazole based UV filters in aqueous samples from Gran Canaria Island (Spain). [1] A. Kabir, K.G. Furtón, Fabric phase sorptive extractors (FPSE), US Patent Application: 14,216,121, March 17, 2014. [2] R. Kumar, Gaurav, Heena, A.K. Malik, A. Kabir, K.G. Furtón, Journal of Chromatography A, 1359 (2014) 16– 25. [3] M.E. Balmer, H.R. Buser, M.D. Müller, T. Poiger, Environmental Science and Technology, 39 (2005) 953-962.

**TH076 Occurrence of PFAS in the European eel (Anguilla anguilla) from the Loire estuary (France)**

M. Couderc, Université de Nantes; I. poirier, MMS EA; A. ZALOUK-VERGNOUX, Université de Nantes; A. Kamsari, Université de Nantes; F. EFFLAND-TROUVE, Marine EEF, France; Y. GNOUX, IRD / MMS EA; B. Le Bizec, ONIRIS / LABERCA; B. Veyrand, ONIRIS; C. Mounayrac, Universitat Catholique de l’Ouest / MMS EA; B. Le Bizec, ONIRIS / LABERCA

The perflourinated and polyfluorinated alkyl substances (PFAS) are largely used as additives for the production of fluoropolymers and as surfactants in industrial and consumer products. The PFAS were basic components in the production of carpets, textiles and packaging products. For these reasons, the Water Framework Directive of the European Commission has set environmental quality standards (EQS) in surface waters (river, lake, transitional and coastal) in 2013. The EQS are the concentrations of pollutant(s) in water, sediment or biota which should not be exceeded in order to protect human health and environment. Among the potential indicator species, the eel may be used. The European eel has specific physiological and behavioral characteristics favorable for the use as chemical sentinel species. As eel pollution profiles were shown to be significantly different along a river at small and large-scale, it would be a valuable fingerprint of the chemical pressure of a specific site, particularly for the lipophilic chemicals. Furthermore since 1950, the eel population has continued to decline. In this context, the pollutant contamination of the Loire estuary was investigated in muscles of European eel (Anguilla anguilla). Yellow eels (n=30) were caught in three different locations along the estuary to highlight variations between sites and sources of contaminations. Silver eels (n=15) were also studied to compare contaminant impregnation between different life stages of the species. Average concentrations in muscles of the eel ranged between: 130-1293 ng/LW for the PFAS. The presence of PFAS and particularly of PFOS in eels from the Loire could be explained by the industrial activities around the estuary (wood transformation, especially paper industry and for metal surface treatment industry). Regarding the spatial and the life stage differences, no significant differences between the PFAS were observed due to the high variability of contaminant levels. These results have supplied new data on the occurrence, levels, and patterns of PFAS in the eels from the Loire estuary and they highlighted the need to further investigations focused notably on the potential effects of these chemicals on this species and their analysis in the water and sediments of the estuary.

**TH077 Selected PPCPs determination in Chilean marine species**

A. Giordano, Pontificia Universidad Católica de Chile; M. Retamal, Universidad de Chile; D. Peñaloza, SP Technical Research Institute of Sweden / Wood Technology; L. Urzua, Universidad Tecnológica Metropolitana

Pharmaceuticals and personal care products (PPCPs) may be detected in diverse environmental matrices and a better knowledge of their occurrence became necessary. This concern has lead to the determination of PPCPs in marine species. Concentration of βestradiol, ibuprofen, carbamazepine along with some sunscreen have been recently measured in Chilean marine products. In particular we analyzed tissues of aurial hake (Merluccius australis), Atlantic salmon (Salmo salar), channel sole (Gadus morhua), and austral ham (Engraulis ringens), particularly fillet and liver from the fish. Samples were collected in Southern Fall 2014 from five fisheries in the IV Region of Chile, extracted by QUECHERS methodology and determined by GC-MS with a previous derivatization step. Analytical issues of the extraction methods and their limitations were examined in order to obtained high recoveries. Preliminary results indicate that levels of PPCPs were very low or not detected for most of the compounds. Final results will be discussed and related with lipid and protein content in the different tissues samples.

**TH078 Australian humpback dolphins (Sousa spp) - Using stranded animals to...**

SETAC Europe 25th Annual Meeting Abstract Book
prioritise PBT analytes in biopsies for critically threatened species

F. Neugebauer, Eurofins GFA Lab Service GmbH / RD; C. Gaas, National Res. Centre for Environmental Toxicology / Public Health Department; N. Lohmann, Eurofins GFA Lab Service GmbH; J.J. Meagre, Queensland Government / Department of Environment and Heritage Protection; O. Paepeke, Eurofins GFA Lab Service GmbH

A concern for human health, the general ecological state of an ecosystem is strongly reflected by animal health, especially for animals at higher trophic levels. The burden of persistent organic pollutants (POPs) and similar compounds in marine animals living in proximity to industrialised coasts is a commonly used indicator of the contamination status of such environments. Especially interesting POPs are the polychlorinated dibenzo-p-dioxins and -furans (PCDD/Fs) and polychlorinated biphenyls (PCBs) which show a high toxicological potency in animals and humans. 2,3,7,8-Tetrachlordibenzo-p-dioxin and PCBs in general have meanwhile been classified as carcinogenic to humans (IARC 2007; 2013). Furthermore, important, since they may be released from ships coatings, is the analysis of organotin compounds (OTCl, as well as typical agricultural chemicals such as persistent organochlorine pesticides, and combustion by-products such as PAHs. In the last couple of years there have been increased stranding incidents of various threatened marine wildlife species along the Australian east coast. Elevated mortality rates of the rare Australian Indo-Pacific humpbacked dolphin (Sousa spp) were observed around Moreton Bay, where 12 stranded individuals (out of an population of estimated ~120 individuals) have been found in 2011–2013. Concern has been raised about their exposure to persistent and toxicologically relevant substances since their stranding rates were much higher than expected. To determine whether contaminant exposure is of concern, and to prioritise which compounds should be monitored in future biopsy sampling, fat and other tissue samples from some of the stranded individuals have been analysed for PCDFs/dl, PCBs, ndl-PCBs, OTC, PAHs and certain organochlorine pesticides. The results indicate high levels of some of these analytes, especially PCBs. Key words: Dioxins, humpback dolphins, PCBs, organotin compounds, PAHs, organochlorine pesticides

TH079
A multibiomarker approach to evaluate caffeine effects on Mytilus galloprovincialis
M. Capuloupo, A. Kiwan, University of Bologna / CBMAB; S. Alberto Ravenna; P. Valbonesi, University of Bologna; E. Fabri, University of Bologna / Bigea Department via Selmi Bologna

The greatest part of the total amount of marketed caffeine (CF) is orally consumed through coffee, tea infusions and other beverages, whereas to a lesser extent is employed therapeutically as active ingredient of a variety of pharmaceuticals and non-prescription products. Various chemicals of both anthropogenic sources and as rapidly as it is dissipated, CF has been found to be one of the most ubiquitous and “pseudo-persistent” compounds within surface waters. It is found in waters worldwide at environmental concentrations comprised in the range of 2-1600 ng/L, with higher values measured in estuarine and coastal waters. Increasing questions have recently been raised as to whether standard eco-toxicology test are able to provide a quantitative insight of the risk associated to CF exposure and its potential impact on aquatic ecosystems. To approach a more sensitive and predictive assessment of ecologically relevant effects triggered by pharmaceuticals on aquatic environment, the use of biomarkers of environmental stress was suggested. The present study aimed at investigating sub-leafal effects induced by exposure to environmentally relevant concentrations of CF, in the Mediterranean mussel Mytilus galloprovincialis, treated with 5, 50 and 500 ng/L CF (nominal concentrations) for 7 days. CF final concentrations were measured in aquaria, and CF accumulation were evaluated in tissues. Through a multi-biomarker approach, lysosomal membrane stability (LMS), along with lipofuscin and neutral lipids content, were measured to assess potential alterations of CF on the Mediterranean mussel. Pro (GST) activities and malondialdehyde tissue accumulation, whereas cypermethrin and deltamethrin levels. The objective of this work was to study the impact of pyrethroid treatments in farmed salmon by comparing their pyrethroid levels and profiles with those of wild salmon. Differences between the possible presentations of the salmon—smoked, farmed, fresh, packed and frozen—were assessed as well. An average daily intake was also estimated and compared to the acceptable daily intake (ADI) of pyrethroids. Over 40 samples of salmon from Norway, Finland, Spain, France, Denmark and Belgium were purchased at retail stores and fishmonger’s stalls in the UK. The analysis of the method monitored 10 pyrethroids, including cypermethrin and deltamethrin. For the sample preparation lyophilized salmon was spiked with internal standards, extracted by sonication and underwent a clean-up with alumina and C18 SPE cartridges. Extracts were analysed by GC-NCI-MS/MS. Method recoveries for the pyrethroids ranged from 94.6% to 98.0% at 0.016 to 1600 ng/L, with higher values. The ADI of the pyrethroids tested concentrations. Overall, present data suggest that there is no significant difference between the pyrethroid profiles of farmed salmon according to origin, species or presence. Average daily intake was estimated for individual pyrethroids considering 94.9 g/day fish consumption for a 67.5 kg person. The estimated values were 4-5 orders of magnitude safely below the AIs.

TH081
Solid bulk cargo shipping - an overlooked threat to the marine environment?
M. Goete, BfR / Transport of Dangerous Goods; N. Mazurek, Bundesinstitut für Risikobewertung BfR / Transport of Dangerous Goods; J. Zeilinger, Institute of Ecopreneurship, University of North-West Switzerland; T. Höfer, Bundesinstitut für Risikobewertung BfR / Transport of Dangerous Goods; H. Maier, Maritime transport is the most important transport sector of the World Economy. In 2013, approximately 9.5 billion tons of goods (more than 90% of world trade) were loaded for seaborne transport in ports worldwide. Solid bulk represents the largest group accounting for more than 50% of all loaded goods. The International Convention for the Prevention of Pollution from Ships, (“MARPOL”) aims to minimize pollution of the seas, including dumping, oil and exhaust pollution. Unlike for liquid bulks such as oils or packaged goods, there has not been any specific anti-pollution regulation concerning dry bulks. The recently amended MARPOL Annex V now prohibits disposal of cargo residues classified as Harmful to the Marine Environment (HME). However, a comprehensive hazard and risk assessment of goods transported in bulk is lacking. The aim of the present study is to analyse whether the transport and the storage of solid bulk cargo represents a threat for the marine ecosystem and/or human health. For this purpose, we analysed publicly available information on discharge quantities, identities and composition of schedules shipped as solid bulk and derive a marine hazard and risk assessment. Currently, it is a routine practice to discharge cargo residues of 60 tonnes and more on board a single solid bulk carrier after unloading. Total inputs of solid bulk into the marine environment were estimated to exceed 5 million tons per year. According to the HME criteria in the MARPOL Annex, the majority of schedules listed in the guidance document for shippers (the IMSBC Code) are classified as non-HME (agricultural and forestry goods, many salts, mineral ores and refined metals). Others potentially meet the HME criteria (chopped or ground plastics or rubber, several ores or alloys containing heavy metals) or have an ambiguous composition (mineral concentrates, scrapmetal, etc.), which hamper identification of its (eco-)toxicological characteristics. Overall, the availability of information necessary for marine risk assessment is limited. Comparatively little attention has been devoted to solid bulk risk assessment by the scientific community. However, we believe the massive inputs in combination with their ambiguous composition and the unclear bioavailability should be seen as an obligation for a better quantification of inputs, exposure and hazard of respective material in order to perform a more conclusive risk assessment.

TH082
Spatial distribution of hydrophobic organic contaminants in bulk marine sediments: a greater threat of effects on the environment than previously thought?
B. Broekmeyer, German Federal Maritime and Hydrographic Agency (BSH); N. Niehus, Hamburg University of Applied Sciences (HAW) / Department of Environmental Engineering; N. Theobald, BSH / Marine Chemistry; G. Witt, HAW Hamburg / Department of Environmental Engineering

The ability of sediments to adsorb organic contaminants is an important factor for the fate of contaminants in the marine environment. The ability of sediments to adsorb organic contaminants largely depends on the grain size of sediments, whereby fine-grained sediment absorbs contaminants more readily than coarse-grained sediment. Additionally, organic contaminants equilibrate between sediment and porewater. This inside partitioning of hydrophobic organic contaminants in marine sediments-porewater systems is of special interest in terms of pollutants bioavailability. The fraction sorbed to sediment is less biologically available for organisms than freely dissolved concentrations in porewater (C_porewater). Therefore, we examined the spatial distribution as well as the inside sediment and sediment-porewater partitioning of 5 polychlorinated biphenyls (PCBs) and 11 polycyclic aromatic hydrocarbons (PAHs) in representative sediments from
gravely sand to sandy mud of the North Sea (German EEZ). Concentrations of PCBs are closely related to the distribution of TOC (R=0.91) and fine grained material (R=0.75). PAH distribution is also affected by grain size (R=0.84) and TOC (R=0.69). Furthermore, the partitioning of PCBs and PAHs between sediment and C_free reveals that lower molecular weight PCBs and PAHs predominate C_free, while higher molecular weight PCBs and PAHs predominate sediment. On a logarithmic scale the sediment-porewater partitioning coefficient correlates significantly (PAH: R=0.92, PCB: R=0.89) with the octanol-water partition coefficient, indicating that the pollutants bioavailability depends on its physicochemical properties, e.g. water solubility.

Challenges for environmental risk assessment of nanomaterials: addressing knowledge gaps, protection goals and regulatory perspectives (P)

TH084 Regulation of Nanomaterials under REACH

Nanotechnology is a rapidly emerging field that involves the manufacture and use of materials at the nanoscale, typically between 1 and 100 nanometers, to create functions, devices, and systems which may have fundamentally new properties and functions. In the EU, NMs are implicitly covered by the substance definition of REACH Regulation 1907/2006 although currently REACH doesn’t explicitly lays down rules about NMs. Until now the problem is still remaining in the way how the specific challenges and unique features existing at the nanoscale have to be tackled and distinctly addressed, given that they may substantially differ from those encountered with bulk materials. As a regulatory agency the European Chemicals Agency (ECHA) plays a proactive role in the international regulatory debate on NMs by providing scientific input and support. To implement REACH, CLP and BPR in the EU, ECHA currently uses two approaches: first being supportive, providing help and advice through webinars, workshops and bilateral discussions with the Registrants of NMs and the second a formal approach where by using the legal instruments already available under REACH (substance dossier evaluation, authorisation and restriction). Under REACH a registration dossier has to be submitted if a substance is produced/imported in volume higher than 1 ton/year and a Chemical Safety Report has to be prepared if the volume is higher than 10 ton/year. The registrant may either chose to register the nanofm in the dossier of the bulkform or submit a specific a seperate dossier for the nanoform. Through a compliance check process potential data gaps can be addressed and the necessary information requested to bring the dossier into compliance with REACH obligation. Thus, through registrant fulfilling the legal obligations in REACH, data and information on the safe use and potential hazards should be generated. Moreover, under REACH, a substance for which concerns have been identified at community level, such substance may be subjected to substance evaluation process. The same reasoning and process may also apply to a nanomaterial or a substance containing nanofoms in the scope of the registration. Under Sub stance evaluation process, Member States evaluate certain substances to clarify whether their use poses a risk to human health or the environment. The objective is to request further information from the registrants of the substance to verify the suspected concern, if necessary.

TH085 Regulatory Challenges in Risk Assessment of Nanomaterials

In October 2014 ECHA organised a Topical Scientific Workshop on Regulatory Challenges in Risk Assessment of Nanomaterials in Helsinki. During this 2-day workshop, ECHA fostered discussions on the possible regulatory impacts of the latest scientific developments in risk assessment of nanomaterials. One of the objectives of the workshop was to identify new or improved approaches in the context of risk assessment of nanomaterials that can be utilised in a regulatory context. Discussions on regulatory challenges were built around five topics addressing current challenges of risk assessment within different regulatory regimes, measurement and characterisation on nanomaterials, metrology and use of different dose metrics in hazard and exposure assessment, environmental hazard and fate assessment, and read-across and categorisation. This presentation will cover the outcome of the discussions of the workshop highlighting issues such as the applicability of specific dose metrics, environmental fate descriptors and characterisation of test methods in hazard and fate assessment, and related research needs from a regulatory perspective. In addition, challenges related to read across and categorisation of nanomaterials will be addressed. ECHA will take on-board the conclusions from the workshop in future work and continue exchanging experiences with EU member states, the scientific community, industry and other stakeholders both in the EU as well as on an international level. It is foreseen that the discussions taken place at the workshop will be considered in the drafting of ECHA guidance for registrants and lead to further actions related to approaches taken in the risk assessment of nanomaterials.

TH086 Dynamic simulation of engineered nanoparticles in the river Rhine
A. Markus, University of Amsterdam / IDEBESS; J. Parsons, P. de Voogt, University of Amsterdam; E. Roex, R. Laane, DELTARES

Nanoparticles can enter the aquatic environment by several pathways, for instance via the effluent of wastewater treatment plants or via/indirect discharge of wastewater. While these are not the only routes, they are probably the most important ones for several common types of nanoparticles. A lot of the many different types of nanoparticles that are being examined for/consideration into commercial and industrial production are actually being used, titanium dioxide, zinc oxide and silver nanoparticles are among the most common. Both titanium and zinc are/undefined in abundance in the environment, either from natural causes or/undefined because of use in traditional products. Silver is somewhat rarer than/undefined the other two, but even that can be found in a typical European river. The question that is investigated here is: can we predict the actual/concentrations of these types of nanoparticles in a river like the Rhine and what is the relative contribution of nanoparticles to the/undefined water quality of such a river? The information on which such a prediction is to be based includes: loads on the total loads of these three metals, derived from/measurements, estimates of the loads of nanoparticles from consumer/production and an understanding of the processes that nanoparticles are/undefined subject to/undefined?/undefined/undefined. The results indicate that nanoparticles can be transported over long/undefined distances, eventually reaching the sea.

TH087 Temporal and spatial trends in engineered nanoparticle mobility and transformations in a watershed model

An adaptation of the USEPA-supported Chesapeake Bay Watershed Model is linked to the WASP7 water quality modeling suite in order to explore the impact of spatial and temporal trends in land use, precipitation, stream flow, sediment transport dynamics, and water quality on the reactivity and mobility of silver (Ag) and zinc oxide (ZnO) engineered nanoparticles (ENPs) released to the James River Basin in Virginia, U.S.A. following sewage treatment. The model compares direct effluent discharges to surface runoff of land-applied biosolids containing realistically transformed ENPs and predicts ENP transport and speciation in river reaches and sediment bed layers over two decades. Fertilizer runoff is shown to account for a significant fraction of total metal stress on loads from ENP sources. Due to high flow-through and bed resuspension in much of the basin, only a small fraction of the total metal mass input to the basin remains at the end of the simulation. However, significant accumulation occurs locally where loads are high and stream velocities are low. Silver from ENPs persists as AgS, whereas zinc exhibits complex speciation. Total metal concentrations from ENP sources fall below USEPA water and sediment quality guidelines in all cases, suggesting that current ENP releases do not pose a significant environmental risk in temperate freshwater systems such as the one considered here.

TH088 Targeting research based on ecological risk assessment needs for nanomaterials
Y. Sultan, N. Khera, N. Davidson, Environment Canada

Environment Canada has been regulating nanomaterials under the new Substances Notifications Regulations (Chemicals and Polymers) since 2007. Over these past 8 years, research in support of ecological risk assessment has been adapted to reflect development in the field. The most recent work was focused on supporting research to feed not only into our domestic programs but also directly into the Organization for Economic Cooperation and Development (OECD) Working Party on Manufactured Nanomaterials (WPMM) Sponsorship Program. Since 2012, Environment Canada’s nanoscience program has been focused on identifying key parameters to predict environmental fate and toxicity. This poster will present the evolution of nano research (i.e., moving beyond method development and focusing on key determinants and categorization frameworks to inform safety) supported by Environment Canada and how it continues to directly impact Environment Canada’s regulatory program.

TH089 Test strategy with regard to the environmental risk assessment of nanomaterials considering regulatory approaches

In the test strategy of a test and environmental risk assessment strategy for
engineered nanomaterials (ENM) addressing the aspects fate and effect. The proposed IME-test strategy and risk assessment approach is a life-cycle oriented one, and thus considers all stages along the life of the ENM. For each single stage it has to be considered whether there is a potential for the ENM to be released into the environment. Furthermore, the initial environmental compartment the ENM is released into has to be identified. If the release potential is negligible, this particular life cycle stage can be neglected. A consideration in case the ENM is released into the environment its durability in the initial compartment has to be screened (tier 0). For that screening any information on the changes or loss of ENM properties is indispensable. In case low durability is ascertained, i.e. the ENM rapidly loses its nano-material properties, the formed chemicals can be handled as conventional chemicals. In case medium to high durability is stated, the first tier of the assessment scheme is entered. On tier 1, both a fate and effect assessment is performed. The assessment results in a predicted environmental concentration in the initial compartment (n-PEC\textsubscript{ini}) and a predicted no effect concentration (n-PNEC). The deduction of n-PEC\textsubscript{ini} needs the information on parameters which determine medium to high durability, and also on the amount of the ENM released as well as on the production volume. It furthermore needs a setting of the size of the initial compartment, e.g., the definition of a local or regional scenario as well as other information such as dispersion stability and PC-properties of the pristine ENM. The effect assessment is based either on screening tests (in case of low production volume) or on tests using OECD test-guidelines (in case of high production volume). Thus, the effect testing on tier 1 comprises two different levels of complexity. Besides n-PNEC-values a classification and product labelling (CPL) can be suggested on the basis of the effect concentration and compared to conventional chemicals, a risk quotient (n-PEC\textsubscript{ini} / n-PNEC) can be formed. The refined n-PNEC-assessment on tier 2 comprises a higher tier testing. The suggested test strategy has been developed based on the knowledge of (inter)national publications and discussions. It also takes into account the conclusions made by the OECD WP5M on ecotoxicity and environmental fate (Berlin, January 2013).

TH90 Toward relevant risk assessments for nanotechnologies: Screening tools and tiered-based testing frameworks
A.J. Kennedy, CEERD-EPRI; S.A. Diamond, NanoSafe Inc. / Midwest Division; Z. Collier, US Army Corps of Engineers / Engineer Research & Development Center; J.G. Coleman, US Research and Development Center / Environmental Laboratory; R. Moser, Georgia Institute of Technology; A.R. Poda, US Army Engineer Research and Development Center; M.A. Chappell, U.S. Army Corps of Engineers / Environmental Laboratory; A. Bednar, US Army ERDC; J.K. Stanley, U.S. Army Engineer Research and Development Center / Environmental Laboratory; J.A. Strickland, U.S. Army Engineer Research & Development Center / Environmental Laboratory

The emergence of engineered nanotechnologies necessitates a better understanding of their potential environmental, health, and safety effects before wide-scale technology deployment. However, the properties of nanomaterials and uncertainty regarding applicable test methods has led to lack of consensus regarding the correct approach or data to use or to apply for testing. While efforts are underway to categorize nanoparticles by their unique properties to form logical management units, procedures for screening technologies and consistent, yet adaptive testing procedures are needed in the interim. ERDC is developing a logical, tiered approach (NanoGRID) for generating the most relevant data to feed risk assessment of nanotechnologies that is inspired by current dredged sediment testing strategies. Briefly, tier 1 consists of categorization of technologies, tier 2 consists of relevant use and release population, tier 3 involves determination of environmental persistence, tier 4 consists of hazard assessment and tier 5 applies case specific investigations. The tiered testing process can terminate once sufficient risk-related information has been collected, or the process provides lines of evidence that the technology in question does not require nano-specific scrutiny. The tiered, adaptive guidance approach allows users to concentrate on collecting the most relevant data, thus accelerating technology deployment while minimizing risk. In addition, we are developing a web-based tool suite (NanoExPERT) that provides fundamental particle fate and toxicity estimation; these screening tools allow visualization of available data to determine relevant toxicity thresholds and assess the environmental data in the suite allow estimation of particle dissolution, DLVO behavior, particle number-density, crystalline face surface area, bioaccumulation factors, and toxicity visualization and screening, a modifying environmental factor function, and a dose metric converter. Each tool is limited only by a database containing bench top or literature data and output is user selectable. These tools are a component of the tiered, adaptive guidance framework.

TH91 Providing comprehensive information on environmental impacts of nanomaterials - The DaNa2.0 Knowledge Base Nanomaterials
D. Kühnel, Helmholtz-Centre for Environmental Research / Bioanalytical Ecotoxicology; K. Nau, C. Mänhardt, Karlsruhe Institute of Technology KIT / Inst Applied Computer Science; H.F. Krug, EMPA; F. Paul, DEChemEA; C. Steinbach, University of South Bohemia in Ceske Budejovice

Assessing the impact of new technologies or newly developed substances on our environment is a challenge, even more so if the applied test methods - both toxicological and analytical - are often found to be inadequate and need amendments as it is in the case of nanotechnology. This is illustrated by numerous publications in the field of nano-ecotoxicology which although they have been investigating the impact of a number of nanomaterials on several organisms almost never allow for explicit statements on potential hazards. This fact not only hampers the knowledge communication to all non-scientists (e.g. consumers) but also complicates the transfer of the obtained results for other scientists. Hence, reliable and understandable information on nanomaterials and their environmental impact is missed, e.g. on the basic questions: What exactly are nanoparticles? What is meant by “exposure”? When do toxicologists speak of a risk? These and many more questions are answered by our web-based knowledge base: www.nanoinfo.info.

In an interdisciplinary approach scientists of the DaNa expert team provide a knowledge base for more transparently wrapping up the results of current research on nanomaterials regarding their influence on humans and the environment in an understandable way. Our presentation of complex scientific data addresses not only the scientific community but is also intended for the broader public, e.g. consumers, journalists, students or scientists from other research areas. In order to facilitate the evaluation process of scientific publications a methodology based on quality criteria was developed. Furthermore, the DaNa-project acts as an umbrella project for current projects funded by the BMBF (German Federal Ministry of Education and Research) with regards to nanotechnology and their ecological benefits. The collected knowledge integrated into the DaNa database could also contribute to the prioritisation of further research needs, DaNa on Twitter: @nano_info 

Kühnel, D. et al. (2014) Environmental impacts of nanomaterials: providing comprehensive information related to a specific form of ENMs. Most of the current knowledge of nanomaterials is still limited as it is in the case of nanotechnology. This is illustrated by numerous articles? What is meant by “exposure”? When do toxicologists speak of a risk? These and many more questions are answered by our web-based knowledge base: www.nanoinfo.info.

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TH92 Closing the gap between exposure and effect assessment of engineered nanomaterials
J.T. Quik, RIVM / DMG; I. Velzeboer, Wageningen University; E. Tsitsiou, Faculty of Earth and Life Sciences VU University / Dept of Ecological Sciences; D. de Zwart, RIVM / Centre for Sustainability Environment and Health; D. van de Meent, RIVM / DMG

For environmental risk assessment a relevant expected exposure concentration is compared with an estimate of the safe concentration of a chemical. For engineered nanomaterials (ENMs) this is usually done with a total concentration of ENMs, e.g. not distinguishing between primary ENMs, aggregates and heteroaggregates. However, it is thought that under environmental conditions ENMs will primarily be present as either aggregates or heteroaggregates attached to natural particulate matter. This is already incorporated in several models that predict exposure concentrations (PECs) in the environment for each of these ENM forms: primary ENMs, ENM aggregates and ENM heteroaggregates. In the effect assessments it is usually unclear whether the resulting effect concentration or other endpoint is related to a specific ENM or ENM aggregate. Furthermore, it is not clear how the PECs are related to PNECs. The question is answered by our web-based knowledge base: www.nanoinfo.info.

TH93 A Nanomaterial by Definition: Ecotoxicology Hazard Characterization and Environmental Risk Assessment of an Organic Pigment
E. Lammer, E. Salmens, BASF SE; U. Veith, BASF Schweiz AG; K. Wiencz, BASF SE

Nanotechnology is regarded as one of the key enabling technologies of the 21st century. To fully realize the benefits of any “new” technology the associated risks to humans and the environment must be addressed. The unique physical properties of some nanomaterials raised concerns that “nano-specific” toxic effects are possible. Despite the increased need for data and information within the last decade focused on nanomaterial safety, specific nano toxic effects have not been identified in animal model systems; the difference is only of quantitative nature. Nanomaterials are chemicals and as with all chemicals some will be toxic at regulatory relevant concentrations and others not. Compared to human health aspects there are very few publications which specifically address the environmental risk assessment of a DPP pigment showing no risk for the environment in various uses. We discuss the challenges facing industry and regulators in assuring the accurate characterization of ecotoxicological hazard and the use of these data in meaningful environmental risk assessments in relation to nanomaterials.
Long-term effects of uncoated silver nanoparticles on the sediment-dwelling polychaete Capitella teleta

M.B. Nielsen; A. Palmqvist, Roskilde University / Department of Environmental Social and Spatial Change; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; A. Gergs, RWTH Aachen University / Department of Environmental Social and Spatial Change

The increasing application of nanoparticles in consumer products has led to increasing concerns about their potential environmental risks. When released to the environment a great proportion of nanoparticles are predicted to end up in the aquatic environment and deposit in the sediment. Knowing the effects of nanoparticles on sediment-dwelling organisms is therefore an important step towards assessing the risks of nanoparticles. Information about the toxic effects of nanoparticles is in many cases still insufficient to carry out proper risk assessments and an example is the lack of long-term ecotoxicological studies conducted with nanoparticles. The aim of this study was to examine the long-term effects of uncoated silver nanoparticles (Ag NPs) and aqueous silver (AgNO₃) added to sediment on survival, growth and reproduction of the sediment-dwelling polychaete Capitella teleta. C. teleta were exposed to three nominal concentrations (1 µg Ag/g dry weight sediment (dw sed), 10 µg Ag/g dw sed and 100 µg Ag/g dw sed) added to sediment as Ag NPs or AgNO₃ for a period of 11 weeks. Uptake kinetics and bioaccumulation of Ag in C. teleta were also studied. Effects at the individual level were extrapolated to predict potential population-level effects using a mechanistic effect model and predictions and their applications for risk assessment will be discussed.

Investigating effects of silver nanoparticles on the soil community - Concept of an outdoor TME

M. Hammonds-Wurtz, A. Toschki, Research Institute gaiac

The production and use of silver nanoparticles (Ag-NP) have markedly increased in recent years resulting in an increasing release of Ag-NP into the environment. The main entry of Ag-NP into the environment occurs via sewage sludge and thus the soil is the compartment receiving the highest load of Ag-NP compared to the aquatic environment. Nevertheless, most studies investigating the potential effects of Ag-NP focused on the aquatic environment, while investigations of effects on soil organisms are scarce. The few studies comprising soil organisms so far have been conducted with single species exposed to Ag-NP for a short-term period. However, there is a lack of knowledge how a complex soil community is affected by Ag-NP over a longer period of time. Ecotoxicological impacts on soil communities can be tested using terrestrial ecosystem (TME). This higher tier approach has been developed to investigate the impact of pesticides on the soil fauna under outdoor conditions over a time period of one year. It has been proven as a valid method to detect both effects on the community level as well as on the population level and to observe a potential recovery during the study period. In our study we will use the TME approach to investigate potential long-term effects of Ag-NP on the soil community. Here we will focus on effects on collembolans and oribatid mites, two important groups among the mesofauna, as well as on the community of earthworms. In this poster the project targets, the study design and the conceptual approach of the investigations will be presented.

Stochastic risk assessment of Ag nanoparticles for inhalation exposure route

C. Pang, University Ca' Foscari Venice / Dept of Environmental Sciences Informatics and Statistics; D. Hristozov, Ca Foscari University of Venice; M. Tsang, US EPA ASPH Fellow / Office of Research and Development; A. Zabed; E. Semenzin, Ca Foscari University of Venice / Department of Environmental Sciences Informatics and Statistics

Silver nanoparticles (AgNPs) are a high-volume substance used in textiles, catalytic, optoelectronic, sensing and biotechnological applications because of their unique antibacterial, optical, electrical and thermal properties. With the increasing application of AgNPs in consumer and medical products, there are growing concerns about their health-related applications. Various studies had showed that AgNPs can migrate to liver, spleen, lungs, kidneys and brain and induce toxicity in vivo. These data demonstrated that reactive oxygen species, oxidative stress and modified inflammatory responses play an important role in in vitro toxicity. Although there is a lot of data on AgNPs toxicity the main shortcomings in the state of the art include a lack of long-term exposure studies essential for risk assessment (in long-term instillation as substitute for inhalation). In addition, mostly acute in vitro and in vivo data are available, while the use of such data to predict long-term risks is not recommended, as suitable material-specific uncertainty factors for exposure duration extrapolation are presently unavailable. Moreover, the pulmonary exposure route has been investigated significantly more than ingestion, which is another important route of exposure, especially in consumer settings. Therefore, we chose the inhalation exposure route to perform long-term health risk assessment of AgNPs. First, a literature review was conducted to collect the available toxicity data for AgNPs. Then, the Benchmark Dose (BMD) approach was applied to derive lower benchmark dose confidence limits (BMDLs) to be used as starting points in estimating Derived No-Effect Levels (DNEL). The BMD method was adopted because it is considered statistically more powerful than the No Observable Adverse Effect Level (NOAEL) method. The last step of the study integrates the obtained DNELs’ statistical distributions with predicted exposure concentrations’ statistical distributions in order to evaluate the risk of AgNPs for inhalation exposure route in a stochastic framework. The proposed stochastic framework is meant to be useful in taking into account inherent uncertainties in the system while not trying to perform a full probabilistic assessment which would require much more data which is hardly collectable. The results from this approach to the AgNPs case study will be presented.

TH97 Chronic effects of CuO nanoparticles on Lymnaea stagnalis: implications for environmental risk assessment?

N. Falk, Roskilde University / Department of Environmental Social and Spatial Change; J. Zwicky, Roskilde University / ENSPAC; J. Reventlow, Roskilde University / Department of Environmental Social and Spatial Change; S.F. Hansen, Technical University of Denmark DTU / DTU Environment; A. Palmqvist, T.B. Knudsen, Roskilde University / Department of Environmental Social and Spatial Change; A. Thit Jensen, F.R. Khan, Roskilde University / ENSPAC; K. Syberg, Roskilde University / Department of Environmental Social and Spatial Change Engineered nanomaterials (ENMs) are increasingly used in a wide range of products due to their specific and advantageous properties. The rapid development within the field of ENMs, has resulted in a demand for regulatory reconsiderations. However, the regulatory ENM risk assessment projects, mainly indoor and outdoor, have not been able to address the challenges regarding particle characterization, lack of test methods and specific guidelines resulting in incomparable scientific data. Further, current regulation is typically derived from acute toxicological data, thereby overlooking the possible chronic effects of ENMs on the environment and human health. In this study chronic effects of copper oxide nanoparticles (CuO NPs) on the freshwater snail, Lymnaea stagnalis, were investigated. Snails were exposed to 2.5, 5.5 and 12 µg Cu/L added as either CuO NPs or CuCl₂ in water for 56 days. Effects on survival, growth rate and reproductive output as well as bioaccumulation were measured. Potential delayed effects and the ability to recover from Cu exposure was further assessed by transferring half the snails to clean water after 28 days post exposure. CuO NPs were characterized by dynamic light scattering, zeta potential, dissolution and TEM. The results are discussed in a regulatory context with regard to the relevance of the existing regulation on ENMs. Focus is on the European chemical legislation REACH, to which extent it is necessary for current methods of environmental risk assessment to encompass the unique properties and specific toxicological effects of nanoparticles.

Comparative effect of silver nanoparticles and silver nanowires on the growth and photosynthetic activity of green alga Chlorococcum infusionum

S. Nam; Y. An, Konkuk University / Department of Environmental Science

Silver nanoparticles (AgNPs) are widely used in various products including medical and coating materials, cosmetics, paints, spray and other products. Silver nanowires (AgNWs) are used in nanoelectronic applications and solar cells. This study investigated the inhibitory effects of polyvinylpyrrolidone (pvp)-coated AgNPs and AgNWs using green alga Chlorococcum infusionum. Chlorococcum infusionum was cultured in Bold’s basal medium (BBM) and exponentially growing strain was exposed to pvp-AgNPs and AgNWs for 72h. Growth and photosynthetic activity of C. infusionum was assessed. Growth inhibition was quantified from yield based on chlorophyll fluorescence, and inhibition of photosynthetic activity was assessed by OJIP chlorophyll fluorescence transient from photosystem II activity. After 72h, growth and photosynthetic activity of C. infusionum was significantly inhibited in both pvp- AgNPs and AgNWs. OJIP chlorophyll fluorescence transient from photosystem II activity remarkably declined with the elevated concentrations of pvp-coated AgNPs and AgNWs. Pvp-AgNPs showed higher inhibition against C. infusionum compared to AgNWs in both growth and photosynthetic activity. This study compares toxicity of pvp-AgNPs and AgNWs exposure on the green alga C. infusionum. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future Planning (2014R1A2A1A1058513).
Food/feed production and packaging. It is expected that these applications will increase in the near future and may therefore become a relevant source of release of nanomaterials (NM) into the environment. To gain more knowledge on this issue, RIKILT and the Joint Research Centre (JRC) have prepared for the European Food Safety Authority (EFSA) an inventory of already marketed and in-development applications of NM in agriculture, feed and food, and carried out a review of regulatory aspects concerning NM in both EU and EU countries. The inventory included the regulatory status based on information collected through an extensive search of literature and webpages, complemented by questionnaires sent to different stakeholders (i.e. industry, research institutes, and competent authorities). An analysis of the information records in the inventory shows that nano-encapsulates, silver and titanium dioxide are the most encountered NM type in agricultural applications. In particular, additive and processing applications outnumber others. The use of NM in preparation/development of novel food, feed additives, and pesticides/biocides is mostly in the research and development stage. In EU, NM are explicitly covered by e.g. Regulation on the Provision of Food Information to Consumers (FIC Regulation 1169/2011), Regulation on Plastic Food Contact Materials and Articles (10/2011), and Regulation on Active and Intelligent Materials and Articles (450/2009). A legally binding definition is also provided in the FIC Regulation. Other pieces of legislation are currently under revision to better address NM, e.g. Novel Food Regulation (258/97) or Annexes to the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation (1907/2006). Countries outside the EU have limited NM-specific legislation and no legally binding definition. They rather adopt a broader approach that builds mainly on the use of exposure assessment for industry and the evaluation of the standard AMPHITOX tests, showed LC50 = 0.08 mg / L and NOEC = 0.08 mg / L.

TH100

Ecotoxicity of graphene-based materials on aeroterrestrial microalgae

A. Montagner, University of Trieste / Life Science; M. Babin, University of Trieste / Department of Life Sciences; M. Prato, University of Trieste / Department of Chemistry; V. León Castellanos, E. Vázquez Fernández-Pacheco, University of Castilla La Mancha / Department of Quimica; M. Tretiach, University of Trieste / Department of Life Sciences; M. Prato, University of Trieste / Department of Chemistry

The importance of graphene-based materials on every day life is currently exponentially rising and hence it is essential to understand the potential effects of this materials-family on living organisms, particularly on photoautotroph organisms, which are at the basis of the primary production of marine and terrestrial ecosystems. In the framework of the Graphene-Flagship project, WP2 “Health and Environment”, we tested the ecotoxicity of two highly standardized types of graphene, few layers graphene (FLG) and graphene oxide (GO), on aeroterrestrial microalgae. Axenic cultures of seven species selected for their different ecology and growth-type were exposed at 0.01, 1, and 50 μg/ml in two ways: in the first one (D, deposited) the graphene suspension was filtered and let to dry out on acetate cellulose membranes, and afterwards an algal cells suspension in water was deposited above the layer of deposited graphene; in the second one (S, shaken), a suspension of algal cells and graphene was prepared, shaken for 24 hrs and deposited on the membranes. Seven inoculated disks (one control plus three D and three S, treated at the three concentrations) were laid on solid BBM at the bottom of special dishes (one control plus three D and three S, treated at the three concentrations) were laid on solid BBM at the bottom of special vessels (n=9 per species) which allow gas exchange in sterile conditions. Vessels were kept at 21°C, 17 μmol photons m−2 sec−1, 12/12 h light/dark photoperiod for 4 weeks, and afterwards membranes were cut in two: one half was used to measure the growth of DM, and the other used for physiological and chemical properties were determined such as: mean size diameter, polydispersivity, zeta potential and encapsulation efficiency during a period of 120 days. Cellular viability was determined using the reduction of 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The number of viable cells (mouse fibroblast) was determined by measuring the amount of MTT converted to formazan (a purple compound) by the mitochondrial dehydrogenases. Solid lipid nanoparticles with fungicides and herbicides presented good colloidal stability as function of the time. The results of cell viability assays showed that the SNL do not provoke a reduction in 3T3 cell viability. The exposure of the cells to the commercial formulations (fungicides or herbicides) showed a strong reduction in 3T3 cell viability (around 25 %) and when the herbicides or fungicides were loaded in SLN the observed cell viability was around 80 %. These results showed that the encapsulation of the pesticides in solid lipid nanoparticles decreases the cytotoxic effects of these chemicals in 3T3 cells and these nanosuspension formulations could be an interesting alternative to control the pests in agriculture and reduce the environmental impacts of non-target organisms. Acknowledgments: Fapesp, Fundunesp, CNPq and Capes.

TH101

Influence of soil pH in the effects of ZnONPs on the antioxidant activities and Zn uptake in three plant species (T. aestivum, R. sativus and Z. mays)

C. Garcia-Gomez, INIA; A. Obrador, ETSI Agronomos (UPyM); J.L. Alvarez, D. Gómez, ETSI Agronomos (UPyM); INIA / Agricultural Chemistry; C. del Rio, INIA; M. Babin, INIA / Dpto. Environment; M.D. Fernández, INIA / Environment

In recent years, the study of phytotoxicity of NPs has made rapid progress, but important issues remain to be solved, among them, the role of soil and the importance of the physicochemical soil characteristics for their toxicity and accumulation potential. It is due to most available information about the NP toxicity in higher plants comes from studies on hydroponic media. Moreover, it is necessary to develop more sensitive and specific toxicity indicators based on biochemical changes for evaluating the toxicity of NPs. The ability to generate reactive oxygen species (ROS) has been identified as a possible mechanism of NP toxicity. ROS accumulation induces lipid peroxidation, membrane and DNA/RNA damage and...
metabolism imbalance. Plants have evolved various protective mechanisms to limit oxidative damage caused by ROS accumulation such as the production of antioxidants (e.g., ascorbate, carotenoids or thiols) and antioxidant enzymes. The aims of this study were to test the influence of soil pH in ZnO NP toxicity and accumulation to different plant species and to assist in the selection of potential biomarkers of soils contaminated with theses nanoparticles. ZnO NPs were selected because there is a growing interest in their use in agricultural formulations taking advantage of their properties as a UV blocker, or as fertilizers. The ZnO NP toxicity was tested in three plant species of agronomic interest: Triticum aestivum, Rabanus sativus and Zea mays. Two agricultural soils: an acidic soil (pH 5.4) and a basic soil (pH 8.3) were spiked with 20, 225, 450 and 900 mg Zn NP kg⁻¹ (Zn basis). The effects of the treated soils on ROS levels and the antioxidative defense system of plants were investigated over 35 days. Thus, the changes on the activity of the antioxidant enzymes (APX, GPX and CAT), the levels of reduced glutathione and proteins, and the effects on lipid peroxidation were measured. To obtain a better understanding of the toxicology of ZnO NPs, these effects were compared with visible damage at the functional level of the whole organism: seedling emergence and growth, and the changes in the chlorophyll levels. In addition, Zn concentration in roots and shoots of plant species were determined.

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**TH104**

**The Effect of Nano-ZnO Ecotoxicity on P. subcapitata**

A. Erdem, Akdeniz University / Department of Environmental Engineering; M. Ozturk, Akdeniz University, National Institute of Operating Zeta Laboratory; C. Moral, Akdeniz University / Department of Environmental Engineering

Nanoparticles (NPs) are the materials with at least two dimensions between 1 and 100 nm. Mostly these NPs are natural products but their tremendous commercial use has accelerated the artificial synthesis of engineered NPs (ENPs). The excessive production and usage of the ENPs have brought a new issue that how the environment (air, water, land) can be affected. Therefore, detail understanding of their sources, release interaction with environment, and possible risk assessment should be provided to form a basis for safer use of ENPs with minimal or no hazardous impact on environment. However, there are still no specific standardized protocols or certified reference materials for the ENPs testing. Nevertheless, researchers are trying to find out the best methodologies to perform these kinds of evaluations. Moreover, the roles of ENPs in the inactivation mechanism of organisms and the properties of the ENPs affecting the ecotoxicity still remain unknown. EU commissions recommend for futher studies on standardization of the methodologies and conducting experiments to assess the behavior and fate of the ENPs in real environmental conditions. In this study, through experimenting with different concentrations of ENPs, we prepared with different dispersion methods in synthetic surface water samples with different water quality parameters on algae P. subcapitata, we have shown that cellular membranes were significantly compromised under ambient light radiation and nano-ZnO concentrations, and even under absence of light. Our results suggest that adverse effects are not necessarily only attributable to individual particles smaller than 100 nm but also to low concentrations of ENPs. Therefore, the PPT (Predictive Protection Threshold) concept was used to support the results of cell viability test. This result enables the understanding of ENP ecotoxicity towards the aquatic environment. The authors would like to acknowledge the financial support from Akdeniz University (AU-BAP Project No 2013.01.0102-010).

**TH105**

**Human toxicity testing strategy towards development for grouping of nanomaterials**

N.M. Delieveeck, Arcadis Belgium / Environment; M. Missaen, Arcadis Belgium nv; E.A. Bleeker, RIVM; D.T. Sijm, Head Bureau REACH; F. Van Broeckhoven, National Institute of Product Quality and the Environment; K. Selles, Ecotox Services Australasia (ESA) laboratory

The use of nanomaterials in manufacturing operations and consumer products has increased as scientists and engineers have realized their benefits. The physicochemical characteristics of nanomaterials may differ from their non-nanomaterial counterparts, affecting fundamental properties as solubility, reactivity, environmental transport and toxicokinetics for both humans or the environment. This study explores the physicochemical properties of nanomaterials and their potential impact on toxicity, kinetics, and exposure in order to come to a strategy towards the development of concepts and criteria for grouping of nanomaterials for the purpose of testing for nanomaterial hazards and determination of potential risks. The proposed testing strategy involves four steps. In a first step, the known information is compiled, including information on potential read-across candidates. Based on this information a hypothesis is developed (step 2) and assessed to determine whether and how a nanomaterial might exhibit unique behaviour under relevant time scales and conditions. To test the hypothesis (step 3) to what extent grouping can be used, a tiered strategy is proposed. In the first tier, physico-chemical data, like chemical composition, surface characteristics (coating, capping agents, functionalization), impurities and particle size are so essential to characterise a nanomaterial that they should always collected. Other data (e.g. shape, porosity), may be appropriate to better understand the potential for exposure. In the second tier, in vitro dissolution/bioaccessibility testing or in vitro reactivity studies to support human health endpoints may be considered. In the last tier, the test strategy may incorporate toxicity testing and/or in vivo toxicokinetic testing. The data and the data collected in the tiers, it can be assessed (step 4) if the nanomaterial has a unique behaviour or not. All the data can be used in a weight-of-evidence approach to support the grouping hypothesis. This exercise was done for the following endpoints: acute toxicity, skin/eye irritation, sensitization, mutagenicity, repeated dose toxicity and carcinogenicity. Silver and titanium dioxide nanoparticles were worked out as test cases.

**TH106**

**Effects of Nanoparticles on Skin Irritation and Corrosion Using 3D Human Skin model**

K. Park, H.Kim, H. Lee, J. Choi, Dongduk Womens University / College of Pharmacy

Effects of nanoparticles including silver (AgNP), titanium oxide (TiO2 NPs), silver oxide (Ag2O NPs) or iron (FeNPs) on skin corrosion and irritation were investigated by using in vitro 3D human skin model, Epiderm(TM). Skin models were incubated with nanoparticles for a definite time period and cell viability was measured by MTT test. Skin irritation was determined by the Draize test: by applying an excess 12h post incubation after washing the test material. In the corrosion test, the viability of Epiderm(TM) treated with positive controls (8N KOH for 3 minutes was 72%). However, the viability for 3-min exposure was 89% with AgNP-treated Epiderm(TM), 96.2% in FeNP-treated group, 95.6% in AINP-treated, and 88.3% in FeNP-treated group, which is over the threshold level. In case of 60 min exposure, viability was over threshold level in all the nanoparticles treated groups. In the irritation test, Epiderm(TM) models were exposed to positive control of 5% SDS for 60 minutes and viability was measured after 42 hr post incubation without test materials. The viability of Epiderm(TM) or AINP were over the threshold level in all the nanoparticle treated groups. IL-1a release and histopathological findings support the results of cell viability test. This results proves the evidence that AgNPs, TiO2 NPs, AlNPs and FeNPs are 'non-corrosive' and 'non-irritating' to the human skin by a globally harmonized classification system.

**TH107**

**Screening risk assessment of nano-enabled products used in restoration of works of art**

E. Semenzin, Ca Foscari University of Venice / Department of Environmental Sciences Informatics and Statistics; C. Pang, University Ca Foscari Venice / Dept of Environmental Sciences Informatics and Statistics; A. Brunelli, Environmental Measurements Laboratory ART (Provincial Government of Venice); T. Balbi, University of Genova / DISTAV; L. Canesi, university of Genoa / DISTAV; D. Chelazzi, University of Firenze; A. Marcomini, University of Venice / Department of Environmental Sciences Informatics and Statistics

Traditional restoration methods and conventional materials often lack the necessary compatibility with the original works of art and a durable performance. To address these issues, the use of nanoparticles (e.g. nanoscale bio-inorganic materials, magnetic properties, catalytic activity, controlled biodegradation, chemical bonding, and preservation of movable and immovable artworks) has focused on the development of manageable methodologies, based on advanced materials with a lower environmental impact, namely nanoparticle dispersions, micellar solutions, microemulsions and chemical gels, to be used for the consolidation, pH control and cleaning of works of art. Environmental impacts of a set of nanoparticles dispersions (i.e. four Ca(OH)2 nanoparticle dispersions in 1-propanol, 2-propanol, ethanol, and two CaCO3 nanoparticle dispersions in water and in a 2-propanol/water mixture) were assessed along their life-cycle by performing a screening risk assessment. Exposure to both humans and the environment was evaluated by estimating the potential nanoparticles release in manufacturing/preparation, application, intermediate post-application stages, thus identifying relevant exposure scenarios. Health and environmental hazards were evaluated by self-classifying the investigated formulations according to the Guidance on the Application of the CLP Criteria provided by European Chemicals Agency (ECHA), supported by tests on nanoparticle dispersions performed according to Human Skin Equivalent Models. The results revealed that nanoparticles are not expected to significantly impact human and environmental compartments. For human health, although the self-classification highlighted potential health hazards for some of the proposed systems, the main route of exposure, represented by dermal contact, could lead to adverse toxicological effects only for one system at the highest tested concentration (representative of real application conditions). All the other systems resulted as not irritant for human skin at concentrations representative of real application conditions. However, the use of common personal protective equipment (i.e. gloves, glasses) and prevention measures (i.e. ventilated room), is always recommended in order assure a complete protection of workers from any potential adverse effects. This is one of the first studies addressing environmental impacts of nano-enabled products that can be
employed for cultural heritage applications, to substitute the use of pure organic solvents and other chemicals with recognized toxicity and environmental impact.

TH108 Conceptual framework for Sustainable Nanotechnologies Decision Support System (SUNDS)

v. subramanian, Ca Foscari University of Venice / Dept. of Environmental Sciences Informatics and Statistics; E. Semenzin, Ca Foscari University of Venice / Department of Environmental Sciences Informatics and Statistics; D. Hristozov, Ca Foscari University of Venice; A. Zabeo 1, I. Malsh 2, Malsch TechnoEvaluation, F. Murphy, M. Mullins, University of Limerick; T. van Harmelen, T. Ligthart, TNO; P. Saling, BASF SF / Sustainability Strategy; I. Linkov, US Army Engineer Research and Development Center; A. Mutela, CAE; A. Mulvany / Department of Environmental Sciences Informatics and Statistics

Vishal sabramanian 1, Elena Semenzin 1, Danail Hristozov 1, Alex Zabeo 1, Ince Malsh 2, Finbar Murphy 2, Martin Mullins 1, Toon van Harmelen 1, Tom Ligthart 1, Igor Linkov 1 3 and Antonio Marcomini 1 3 1 Department of Environmental Sciences, Informatics and Statistics, Ca Foscari University of Venice, Italy 2 Malsch TechnoEvaluation, Utrecht, The Netherlands 3 Kemmy Business School, University of Limerick, Ireland 4 TNO, Utrecht, The Netherlands 4 US Army Engineer Research and Development Center, Concord, MA, USA Track and session: Track G: Prospective Life Cycle Thinking approaches for the definition and implementation of sustainability strategies in industry and policy making

Keywords: Sustainable Nanotechnology, Decision Support, Multi Criteria Decision Analysis Application of methods like Life Cycle Assessment (LCA) and Risk Analysis (RA) to Engineered Nanomaterials (ENM) are challenging because of knowledge and data gaps, and can impede safe and sustainable nanomanufacturing. While LCA has great utility for quantitative impact evaluation and regulatory and industrial decision making, lack of nanomaterial-specific characterization factors and reliable Lifecycle Inventory data. Multi-Criteria Decision Analysis (MCDA) can be used to integrate RA and LCA within a framework for Sustainable Nanotechnology that can also incorporate stakeholder values and estimate uncertainty. The European Commission has funded a project on sustainable nanotechnology (SUN, http://www.sunnfp7.eu/) that aims to build tools to assess ecological and human health risks, environmental impacts, risk management measures and benefits of nanoproducts. These tools will be integrated within an overarching decision framework and support tool for Sustainable Nanotechnology to support the selection of risk management alternatives (e.g. safety by design technological alternatives, personal protective equipment). The SUN Decision Support System (SUNDS) is currently in development in the SUN project through a process of integrating SUN tools with user needs. The framework will be implemented in a user-friendly and web-based tool to be tested on the SUN case studies. We present SUNDS conceptual framework and user needs (industry, regulatory and insurance sector) with respect to SUNDS features.

TH109 The GUIDEnano strategy for nanomaterial environmental hazard assessment ab initio

G. Jane, Acondicionamiento Tarrasene; M. Diez-Ortiz, Lietat Technological Center; M. Fernandez-Cruz, INIA - National Institute for Agricultural and Food Research and Technology / Environment; D. Hernandez-Moreno, INIA / Environment; J. Izquierdo, INIA; D. Spurgeon, Centre for Ecology & Hydrology; M. Park, RIVM; N. Ferraz, University of Uppsala; J. Catalán, Finnish Institute of Occupational Health; F. Lopez-Campillo, ISMAR / Sustainability; C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology GUIDEnano aims at developing an interactive web-based Guidance Tool to support the risk assessment and risk management of a nano-enabled product. A first step will be to assess the possibility to use existing hazard assessments for similar materials, by assessing similarity between the exposure-relevant and the already tested NMs. This will be done by using a set of predefined read-across criteria, such as chemical composition, degradation/dissolution rate, shape, primary size and aggregated size, charge, and different coating properties. The consideration of all these read-across criteria leads to a similarity score for each physicochemical property evaluated. To determine the ultimate similarity score between two NMs, the highest similarity score is selected. As a second step, in cases where the exposure-relevant material is not sufficiently similar to any of the previously assessed materials, the Tool would base the hazard assessment on individual studies available for the NM of interest. Each individual study, providing it is relevant for the environmental compartment of interest, would be evaluated for two aspects: its reliability and the similarity of the tested NM to the exposure relevant NM (i.e., the read-across). In the process, the reliability grade and the similarity grade are combined in a single score based on the Klimisch score and a specific nano score that accounts for the nanomaterial characterization details included in the study. A minimum score for each individual study will be used to define the final PNEC value and, in such cases, the score will also inform on the uncertainty around it. A third and final step, in cases where no relevant test information would be available for a nanomaterial, we consider the use of general categories of nanomaterials and associated default hazard values. There is so far little evidence of direct physical effects of NMs in the environment, and the vast majority of data suggests that observed toxicity can be explained by toxicity from the component materials. Hence, we consider that, with the current state of knowledge, an expected conservative default value for a PNEC would be based on the toxicity of the NMs component material.
toxicogenomics-based assay presents a promising alternative for fast, efficient and mechanistic genotoxicity screening and assessment of drugs, foods, chemicals, nanomaterials and environmental contaminants.

THI12  
Colon carcinogenicity assessment of water and effluents under the influence of textile activities  
F.H. Fernandes, UNIVERSIDADE ESTADUAL PAULISTA - UNESP / Pathology; G.d. Umbuzeiro, FACULTY OF TECHNOLOGY - UFMG / LEAL; M.O. Nascimento, A.C. Sávio, C.C. Munari, B. Anfílio, Universidade Estadual Paulista UNESP / Pathology; D.F. Salvadori, Unesp

Az o dyes belong to a large family of synthetic dyes that are highly resistant to natural degradation. They are widely used in the textile industry and can reach the aquatic environment. We evaluated the potential of samples collected at two sites of two both under the influence of textile discharges to induce colon carcinogenicity in rats. Samples were collected during a severe drought condition (February, 2014), (i) at Quilombo River (QR) and (ii) and the treated effluent of Carioba Wastewater Treatment Plant (WWTP), Americana, State of Sao Paulo, Brazil. Rats (12 per group) were daily treated with 30 mg/l in QR. SDS, WWTP treatment was carried out with different samples at 0.1, 10.0 and 10.0 % concentration by drinking water during two weeks. Animals treated with ethylenediamine tetra acetic acid (EDTA, 1.85 mg/kg body weight) and with 1,2-dimethylhydrazine (DMH, 160 mg/kg b.w.) administrated by subcutaneous injection twice a week for two weeks were used as negative and positive controls, respectively. After six weeks of treatment, animals were euthanized (protocol approved by the Ethical Committee for Animal Use). Anatomical features (hearth, kidney and liver weight) were also assessed. One-hundred sequential fields from proximal, medial and distal colon were analyzed for the presence of aberrant crypt foci (ACF). Liver hypertrophy was observed in rats treated with both WWTP and QR samples. Increased frequency of ACF in the proximal colon was detected in those animals treated with WWTP at the two highest doses (1 and 10%; p< 0.05). In conclusion, data suggest the presence of potential carcinogens in Quilombo river and WWTP treated effluent samples.

THI13  
Chemical characterization and toxicological evaluation of airborne PM10, PM2.5, and PM1 near cement plants  
N. Nogueira, Universitat Rovira i Virgili; F. Sánchez Soberón, Universitat Rovira i Virgili / Chemical Engineering; J. Rovira, Departament d Enginyeria Quimica; M. Mari, Rovira i Virgili University / Chemical Engineering; J. Sierra, Universitat Rovira i Virgili / Soil Science Unit; E. Marti, Universitat de Barcelona / Products Nucleolitica Biologia Vegetal e Edafologia; M. Nadal, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; J.L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering

Urban particulate air pollution has a major impact on the quality of life and human health. There is a special concern over the amount of suspended fine and ultrafine matter in the ambient fine particulate matter (PM) is often referred to as PM<sub>2.5</sub> and PM<sub>1</sub> as target cells. The results show that Microtox is an excellent screening test to evaluate the potential of toxicity and genotoxicity was assessed by means of soluble salts were analysed in the three different particles fractions. The size, morphology and chemical composition of particles were also observed by means of chromatography. The objectives of this study are to characterize the size, morphology and chemical composition of the atmospheric particles (PM<sub>10</sub>, PM<sub>2.5</sub>, and PM<sub>1</sub>) in two areas (urban and rural) located close to a cement plant. Their potential of ecotoxicity, cytotoxicity and genotoxicity for the human health is also determined. Samples of PM<sub>10</sub>, PM<sub>2.5</sub> and PM<sub>1</sub> were fractioned with high-volume active sampling devices, and the contents of mineral matter, trace elements, C, TOC, N, S and soluble salts were analysed in the three different particles fractions. The size, morphology and composition of particles were also observed by means of Environmental Scanning Electron Microscope techniques, in order to observe differences between different PM fractions. The levels of ecotoxicity were evaluated by using the photo-luminescent bacteria Vibrio fisheri, while the potential of toxicity and genotoxicity was assessed by conducting the MTT and Comet assays, respectively. The results show that human lung epithelial cells A549 as target cells. The results show that Microtox is an excellent screening test to perform a first evaluation of air quality. In addition, A549 cell lines allowed to distinguish when air samples are toxic and/or probable carcinogens for cell lungs.

THI14  
Electrolyte-peptide adductome - Relationship with site- and mechanism-specific reactivity  
C. Siwiak, Helmholtz centre for environmental research - UFZ / Ecological chemistry; C. Rickmeyer, Ilmenau University of Technology; S. Hirth, Martin-Luther-University Halle Wittenberg / Institute of Chemistry Food Chemistry and Environmental Chemistry; A. Böhme, UFZ - Helmholtz Centre for Environmental Research / Ecological chemistry; G. Schuurmann, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry

For unraveling mechanistic causes of disease formation, the exposome defined as cumulative totality of exposure in the organism contains pertinent information. Importantly, this internal chemistry is affected not only through the uptake of exogenous compounds, which is often studied by the endogenous generation of substances in response to external stress, with electrolyte aldehydes generated from lipid peroxidation forming one prominent example. Because electrophiles are ready to react with nucleophilic sites of peptides, proteins and the DNA, the respective adduct formation contributes significantly to the adductome as part of the exposome. In the presentation, we compare peptide adducts of organic electrophiles as determined through LC/MS-MS with their mechanism- and site-specific reactivities, the latter of which are quantified through kinetic chemosassays employing mono- and bifunctional model nucleophiles. The discussion includes the scope of electrophilic reactivity profiling for predicting electrophile-protein adductome patterns, taking into account opportunities for calculating the latter from molecular structure. [1] Rappaport SM, Smith MT 2010. Environment and disease risks. Science 330: 460-461. [2] Jacobs AT, Marnett LJ 2010. Thiol reactivity and its impact on the cili-ate toxicity of a,b-un-saturated aldehydes, ketones and esters. Chem. Rev. Toxicol. 23: 1905-1912. [6] Thaens D et al. 2012. Chemosassay screening-ing of DNA-reactive mutagenicity with 4-(4-nitrobenzoxy)pyridine – Application to epoxides, oxetanes and sulfur heterocycles. Chem. Rev. Toxicol. 25: 2092-2102. [7] Wondrusch D et al. 2010. Local electro-phili-predicts the toxicity-relevant variety of Michael acceptors. J. Phys. Chem. Lett. 1: 1605-1610.
0.5 ng mL\(^{-1}\) and extraction recoveries from 71-134\% for all congeners. Intra- and inter-assay coefficients of variation were between 3.8 and 15\%. In order to evaluate the applicability of the method, a preliminary evaluation (N=90) was conducted in plasma from occupationally exposed workers of a transformer recycling plant and residents exposed to very high levels of PCBs in a German cohort. High chlorinated (penta- through hepta-chlorinated) OH-PCBs were the predominant congeners in humans, with concentrations up to 44.2 ng mL\(^{-1}\). Lower chlorinated OH-PCBs were occasionally detected, although monochlorinated OH-PCB congeners was not present in any of the samples. Unknown OH-PCB congeners peaks were also frequently present in the samples at relatively high concentrations, highlighting the need for the development of new standards. In addition, the relationship and associations between OH-PCB metabolites and their parent compounds in the studied cohort were also assessed.

**TH117**

**Targeted and Non-targeted Analysis of Phthalate Metabolite in Human Urine with Liquid Chromatography Mass Spectrometry: Identification and Occurrence for Exposure Assessment**

Y.-F. Fang, A. C. Yao, Health Canada; P. Chan, McGill University

Phthalate esters are ubiquitous in our living environment due to their widespread use. The human exosompe represents the totality of the chemical exposures over the course of an individual’s lifetime. It is a challenge to assess human exposure to chemicals like phthalates through direct measurements of environmental contaminations levels since they may not reflect actual exposure in an individual. As a result, a more accurate assessment of the exposure of the population to phthalates has been attempted by a combination of measuring specific metabolites of the phthalates in urine or other biological samples and information on the sources of phthalates, bioavailability and metabolism pathways in humans. In this study, we show the use of liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF) to analyze the targeted screening and identification of unknown phthalate metabolites in human urine. The precursor ion scan technique in triple quadrupole mass spectrometry was used to screen any molecule that can produce a product ion, m/z 121. Phthalate metabolites have a common product ion [C\(_{n}\)H\(_{2n}\)OOC\(_2\)] which is represented as m/z 121 in the mass spectrum. In the quadrupole time-of-flight mass spectrometry, which is high resolution mass spectrometry (HRMS), was utilized to elucidate structural information of the peaks observed in the precursor ion scan. The high mass accuracy and exceptional tolerance for sample complexity inherent to HRMS instruments allows distinguishing of analyte ions and product ions from interferences with similar mass and identification of unknown metabolites at trace level. A core-Valencia-Shell column was used to further enhance the specificity of identification for non-targeted metabolites through improving the separation efficiency on the column prior to mass spectrometry. Canadian male urine samples in two groups, control and infected, were subjected to a non-targeted analysis to identify any new or unmonitored phthalate metabolites of potential concern using a combination of LC-MS/MS and LC-QTOF. Targeted analysis was undertaken only using metabolites identified in this method. This technique possesses superior separation capabilities, fast screening of phthalate metabolites and accurate identification of unknown metabolites.

**TH118**

**Influence of socio-demographic and diet determinants on the levels of mercury in preschool children from a Mediterranean island.**

M. Garrí, IDAE-CAIC / Environmental Chemistry; J.O. Grimalt, Environmental Chemistry; M. Torrent, Área de Salud de Menorca IB-Salut. Fundació Caubet-CIMERA; J. Sanver, Centre for Research in Environmental Epidemiology

Mercury is well known as a major persistent pollutant. It can cause severe neurological damage to children. Humans are exposed to mercury basically through fish consumption. This study focuses on the presence of mercury in the infant diet in preschool children from a Mediterranean island.

The determination of total mercury (THg) in hair was performed using inductively coupled plasma mass spectrometry (ICP-MS). A number of contaminants were detected, which is high resolution mass spectrometry (HRMS), was utilized to elucidate structural information for non-targeted analysis to identify any new or unmonitored phthalate metabolites of potential concern using a combination of LC-MS/MS and LC-QTOF. Targeted analysis was undertaken only using metabolites identified in this method. This technique possesses superior separation capabilities, fast screening of phthalate metabolites and accurate identification of unknown metabolites.

**TH119**

**Human exposure to PAHs bound to PM fractions in an area influenced by a cement plant. PBPK application and risk assessment**

E. A. Montero Soberón, Universitat Rovira i Virgili / Chemical Engineering; M. Mari, Rovira i Virgili University / Chemical Engineering; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering; J. Rovira, Departament d'Enginyeria Química; M. Nadal, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; V. Kumar, Universitat Rovira i Virgili / Chemical engineering department; J.L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health

Cement production is an energy intensive process that requires huge amounts of materials and energy. As a consequence of the combustion performed inside a cement kiln, a number of pollutants may be generated and released to air, being polycyclic aromatic hydrocarbons (PAHs) one of the most typical families of chemicals. PAHs form a varied group of chemical compounds structurally characterized by PAHs among aromatic fragments. In order to be found, they can be identified as vapor or linked to air particulate matter (PM). Inhalation is the main direct pathway of exposure to PAHs. Once inside the human body, PAHs may act as carcinogenic precursors, as well as being able to generate pulmonary, gastrointestinal and renal diseases. So far, gaseous PAHs have been more extensively studied. However, greater attention is being paid nowadays in elucidating the occurrence of particle-bound PAHs, since persistence and toxicity could be higher. Although it has been reported that PM bound to PAHs are usually associated to particles smaller than 1-2 μm, studies analyzing PAHs contained into this fraction are still sparse. Moreover, there is no much information about PAHs in areas influenced by cement plants, since information has been restricted to other chemicals, such as dioxins and furans, heavy metals and inorganic gases. Furthermore, the subsequent adverse health effects for the population living in the vicinity of these facilities is being subject of study. This study is aimed at understanding the distribution of PM-bound PAHs in an area under the direct influence of the emissions of a cement plant. Three PM fractions (10, 2.5 and 1 μm) were sampled with high volume active samplers in sites located near a cement factory in the Catalanía (Spain). The airborne PAHs were extracted from the sampling filters by means of HRGC/HRMS to elucide the PAHs content. In order to estimate the PAH exposure, a human respiratory tract model linked to a physiologically based pharmacokinetic model (PBPK) was used. Subsequently, human health risks were assessed, highlighting the risk of cancer associated to the inhalation of PAHs. The results of our study will help understand the distribution, target organs and health risks associated to PAHs among vulnerable groups, such as children or the elderly, who are exposed to PAHs through emissions of a cement factory. Hence, information might be applied to areas locally impacted by other industries which potentially emit PAHs to air, such as oil refineries.

**TH120**

**Development of a personal exposure model based on Agent Based Modelling**

D.A. Sarigiannis, E. Handakas, D. Chapizanis, V. Kumar, Universitat Rovira i Virgili / Chemical Engineering; M. Nadal, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; J. Rovira, Departament d’Enginyeria Química; M. Mari, Rovira i Virgili University / Chemical Engineering; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering; J. Rovira, Departament d’Enginyeria Química; M. Nadal, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; V. Kumar, Universitat Rovira i Virgili / Chemical engineering department; J.L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health

Toxicology and Environmental Health; V. Kumar, Universitat Rovira i Virgili / Chemical engineering department; J.L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health

Cement production is an energy intensive process that requires huge amounts of materials and energy. As a consequence of the combustion performed inside a cement kiln, a number of pollutants may be generated and released to air, being polycyclic aromatic hydrocarbons (PAHs) one of the most typical families of chemicals. PAHs form a varied group of chemical compounds structurally characterized by PAHs among aromatic fragments. In order to be found, they can be identified as vapor or linked to air particulate matter (PM). Inhalation is the main direct pathway of exposure to PAHs. Once inside the human body, PAHs may act as carcinogenic precursors, as well as being able to generate pulmonary, gastrointestinal and renal diseases. So far, gaseous PAHs have been more extensively studied. However, greater attention is being paid nowadays in elucidating the occurrence of particle-bound PAHs, since persistence and toxicity could be higher. Although it has been reported that PM bound to PAHs are usually associated to particles smaller than 1-2 μm, studies analyzing PAHs contained into this fraction are still sparse. Moreover, there is no much information about PAHs in areas influenced by cement plants, since information has been restricted to other chemicals, such as dioxins and furans, heavy metals and inorganic gases. Furthermore, the subsequent adverse health effects for the population living in the vicinity of these facilities is being subject of study. This study is aimed at understanding the distribution of PM-bound PAHs in an area under the direct influence of the emissions of a cement plant. Three PM fractions (10, 2.5 and 1 μm) were sampled with high volume active samplers in sites located near a cement factory in the Catalanía (Spain). The airborne PAHs were extracted from the sampling filters by means of HRGC/HRMS to elucide the PAHs content. In order to estimate the PAH exposure, a human respiratory tract model linked to a physiologically based pharmacokinetic model (PBPK) was used. Subsequently, human health risks were assessed, highlighting the risk of cancer associated to the inhalation of PAHs. The results of our study will help understand the distribution, target organs and health risks associated to PAHs among vulnerable groups, such as children or the elderly, who are exposed to PAHs through emissions of a cement factory. Hence, information might be applied to areas locally impacted by other industries which potentially emit PAHs to air, such as oil refineries.
b) differences in activity intensity. Overall, exposure to PM and especially deposition across the HRT are higher for children, as a result of the higher fraction time spent outdoors and in transport, the higher bodyweight-normalised inhalation rate, the more intense overall activity and age-dependent differences in respiratory physiology.

TH121  Are fine particles the most dangerous? An exposure and dosimetry approach
F. Sánchez Soberón, Universitat Rovira i Virgili / Chemical Engineering; M. Mari, Rovira i Virgili University / Chemical Engineering; V. Kumar, Universitat Rovira i Virgili / Chemical engineering department; M. Nadal, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; J.L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering
Particulate matter (PM) is a complex mixture of extremely small particles and liquid droplets suspended in the atmosphere. Small particles in ambient air are originated from a wide range of sources (such as traffic, industry, energy production or domestic combustion). The inhalation of PM causes many adverse health effects on the population, to control their levels, PM outdoor concentrations, especially PM10 and PM2.5 fractions (those smaller than 10 and 2.5 μm of diameter respectively), are widely monitored. However, in developed countries population spend most of their time indoors. Data regarding indoor aerosol particles are not usually available, so “Indoor Aerosol Modelling” can be used as an alternative to estimate indoor levels. Apart from PM levels, health effects caused by PM depend on its physical and chemical properties. Size plays a key role determining the part of the respiratory tract where the particles deposit and, therefore, their potential of being harmful. The objective of this study was to evaluate the exposure, indoor and outdoor, to different fractions of airborne particles and assess the dose retained in the different parts of the human respiratory tract. To do that, outdoor concentrations of three PM fractions (10, 2.5 and 1 μm) were measured in Mataró (Barcelona, Spain), an area with many industrial activities (a cement plant among them). The outdoor concentrations were used to estimate indoor levels of PM fractions by means of Indoor Air Quality (IAQx) model (US EPA) taking into account the time activity patterns for different groups of population. In turn, Muliple Path Dosimetry (MPPD2.11) model was used to determine the internal exposure and the distribution of different PM fractions in the respiratory tract. The results showed that the pattern of exposure to particles changes significantly when considering different activities and indoor concentrations. Conclusions of this study may be a key factor for a better understanding of biological response to PM exposure and, hence, the human health risk assessment.

TH122  Exposure assessment of airborne endotoxin, glyphosate residues, and particles in farms during summer spray season
A. Adhiakari, Georgia Southern University / Department of Environmental Health Sciences
Many herbicides – molecular weight chemicals including some pesticides and herbicides are capable of inducing occupational asthma. Glyphosate [N-phosphonomethyl glycine] is one of the most commonly used broad spectrum nonselective herbicides in the world. Glyphosate application was associated with asthma and rhinitis in large-scale epidemiological studies of US farmers and a possible role of glyphosate in airway inflammation was previously shown in our laboratory study. Levels of airborne endotoxin and dust particles in farm environments are typically high as the body. The cumulative exposures to endotoxin, glyphosate residues, and dust particles in farms could be associated with airway inflammation and asthma among farmers. In this field study, we have examined parallel exposure levels of airborne endotoxin, glyphosate residues, and particles of different sizes in several large corn farms of U.S.A. during summer glyphosate spray seasons. Samples were collected by conventional and non-conventional size-selective aerosol sampling/source testing methods (for aerosolizable contaminants from ground surface) before and during/after spraying. Extracts of aerosol samples were analyzed for endotoxin and glyphosate by Limulus amebocyte lysate assay and glyphosate-specific ELISAs. Higher levels of endotoxin and glyphosate were observed in >1.8 μm particle size fractions and total inhalable aerosols compared to other particle size fractions. Endotoxin levels in air were found to be same during pesticide spray and in control samples. Rereroasolizable glyphosate residues and endotoxin on farm soil surfaces after spray were mostly below the detection limits. Glyphosate spraying in all farms increased particle concentrations for all sizes (0.3 - >20 μm), however, the increasing trends were more prominent for particles of >3 μm sizes. Action levels for PM10 are supported by WHO and NIOSH 2013.16 from the National Institute of Environmental Health Sciences (NIEHS). The action levels are the responsibility of the authors and does not necessarily represent the official views of the National Institute of Environmental Health Sciences or the National Institutes of Health.

TH123  Environmental risk assessment for human exposed to airborne silica dust in ceramics manufacturing
Y. Liu, B. Wu, Y. Cheng, Y. Shu-Han, National Taiwan University / Department of Bioenvironmental Systems Engineering; N. Hisieh, Department of Bioenvironmental Systems Engineering; C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering
Airborne pathogenic dust of silica (SiO2) is posed to be one of the greatest threats to human health effects, causing pulmonary fibrosis (silicosis), lung function deficits, pulmonary inflammation, and lung cancer. The purpose of this study was to assess human health risk exposed to airborne silica dust in Taiwan ceramics manufacturing. We conducted the dose-response models for describing the relationships between exposure dose and inflammatory responses, by which health risks among workers can be inferred. We found that silica contents were 0.22 – 33.04% with concentration ranges of 100 – 533.66 and 0.46 – 1763.80 μg m\(^{-3}\), respectively, in tile and commodity ceramic factories. We showed that granulation workers in tile ceramic factory had the highest total silica lung burden (~1000 mg) with a cumulative silica lung burden of ~4 × 10\(^{10}\) mg-yr. The threshold estimates that workers are exposed to are sufficient to have an effect on human health for inflammation and fibrosis are 407.1 ± 2.77 × 10\(^{-10}\) (mean ± sd) and 505.91 ± 2.31 × 10\(^{-9}\) mg, respectively. For granulation workers, a long-term of 30 – 45 yr exposure to airborne silica dust was likely to pose severe adverse health risks of inflammation and fibrosis. We suggest that proprietors should provide health examination to prevent the associated occupational disease attributable to long-term exposure of inhaled silica dust.

TH124  Evaluation of the Exposome: Non-targeted screening analysis of environmental contaminants in human urine by liquid chromatography coupled to high resolution mass spectrometry
A. Cortéjade, A. KISS, Institut des Sciences Analytiques / TRACES Team; C. Cren, Institut des Sciences Analytiques UMR TRACES Team; E. Vuillot, CNRS; A. Buler, Institut des Sciences Analytiques UMR TRACES Team / Service Central d’Analyse
The impact of the environment on human health has been conclusively demonstrated. In recent decades, scientists have shown that many chronic diseases are related to our environment. In this context, a new term was born in 2005: the exposome. It corresponds to all types of exposures humans are subjected throughout their lives via lifestyle, diet, and social environment, as well as the body responses to these exposures. The exposures the man is facing are numerous and all environmental contaminants present in everyday life are part of the Exposome. So the concept of the Exposome highlights the need to develop measurement methods to evaluate human exposures. Furthermore, the Exposome is an unstable concept that is changing over time. Therefore, it is necessary to develop, establish and evaluate routine measurement methods. In this context, a method was developed on crude urine and on a LC-ToF instrument to detect metabolites and degradation products of known or even unknown contaminants in contact to man in daily routine by a comprehensive approach. The developed analytical strategy consists in a broad screening of the urine based on the exact monoisotopic mass of the environmental contaminants, their metabolites and degradation products contained in urine. The urine appears to the preferred biological matrix for studying the Exposome: it is easier to obtain (non-invasive, readily available) and includes a large number of endogenous and exogenous metabolites. This study focuses on the comparison of urine mapping from different cohorts with the aim of further studying the Exposome and upgrading the databases. The implementation of this tool to measure the Exposome associated with cancer studies are easier and more easily to understand the causal relationships between diseases and environmental factors. Keywords: Exposome, LC-HRMS, urine, screening.

TH125  Improving the design of biomonitoring studies for persistent organic pollutants: Insights from population based pharmacokinetic modelling
T. Bui, IVL Swedish Environmental Research Institute Ltd / Natural Resources Environmental Effects; A. Palm Cousins, IVL Swedish Environmental Research Institute Ltd; M. MacLeod, ITM - Stockholm University / Dept of Environmental and Analytical Chemistry ACES; I. Mueller, Exonto / National Research Centre for Environmental Toxicology Exonto
Persistent organic pollutants (POPs) have been of great concern over the last few decades. Various biomonitoring programmes have been developed on a national or international scale to monitor the levels and trends of POPs in human populations. However, many challenges have to be faced when planning and conducting a biomonitoring study, for instance sampling (recruitment, matrix to sample sampling frequency, area of sampling), storage and analysis (pooled or single samples) and data evaluation. Since these studies can be very expensive, cost-efficient strategies are required. The aim of this work is to provide guidance on how to design a biomonitoring study to assess time trends of POPs in the general human population. Recommendations regarding cost-efficiency are offered and tools for evaluating the effectiveness of an action (e.g. restriction) to reduce exposure are presented. A key tool used in this study is the one-box, dynamic pharmacokinetic model developed by Ritter et al. in 2009. This work distinguishes between two important phases during a typical timeline of a POP: 1) Pre-action or increasing exposure phase and 2) Post-action or decreasing exposure phase. During
the pre-action phase, body burdens showed no differences between younger and older adults. Therefore, cross-sectional sampling of a population is not required. In the post-action phase, simulations show different results depending on the intrinsic elimination half-life of a chemical. Rapidly eliminated chemicals showed the same pattern as in the pre-action phase, but clear age-concentration relationships could be observed for chemicals with slow elimination rates, where older adults have surprisingly higher body levels than young adults. Since intrinsic elimination is mostly used to sample cross-sectional during this phase in order not to potentially underestimate body levels for older adults. During this phase, it is also possible to evaluate the effectiveness of an action by estimating turn-off ages (the age where concentrations reach a plateau and cease to increase) in cross-sectional data. Modelling results revealed a key turn-off age of 25 ± number of years after peak exposure, below which more aggressive actions to reduce exposure will likely result in lowering the overall body burden of the population further. At turn-off ages equal or higher than that value, further actions have no significant effect on the population.

TH126

Comparative exposure to DEHP from food contact materials: application of the product intake fraction
A.S. Ernsto, Quantitative Sustainability Assessment; O. Jolliet, University of Michigan / Environmental Health Sciences School of Public Health; P. Fanke, Technical University of Denmark / Quantitative Sustainability Assessment
Food contact materials (FCM), e.g. bottles and food handling gloves, can contain potentially toxic substances, such as Toxicology and Environmental Health (DEHP, CAS: 117-81-7). To investigate the contribution of FCM to dietary DEHP exposure we apply the product intake fraction (PiF, g intake/g in product) – a metric accounting for human intake of chemical mass per unit of mass embodied within a product. PiF of ingestion of DEHP is estimated from empirical data for PET water bottles on the order of 1E−7 through 1E−5 and for food handling gloves from 1E−6 to 1E−2. The key uncertainty is related to underestimation of chemical content in FCM, circumstances of use (e.g. food item), and any analytical uncertainty. Using PiF, maximum allowable concentrations of DEHP within water bottles and gloves were calculated with respect to regulatory thresholds. A hypothetical average PiF for the FCM sector was calculated via production volume and oral exposure doses estimated from NHANES data. In both cases the indication was gloves may contribute more to DEHP exposure when used with certain food items than bottled water. DEHP content in gloves greater than 5% would cause exceedance of US EPA threshold when used with certain food items, e.g. radishes based on PiF calculated here. The PiF used in this context has applications for regulations related to FCM and exposure assessments on a per unit kilo basis.

TH127

Tissue dosimetry modeling of chemical mixtures containing Metals: a case study of Cd, Hg and Pb in humans
V. Kumar, Universitat Rovira i Virgili / Chemical engineering department; M. Nadal, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; J.L. Domingo, Universitat Rovira i Virgili / Laboratory of Toxicology and Environmental Health; S. Karaktsios, A. Gotti, D.A. Sarigiannis, Aristotle University of Thessaloniki / Chemical Engineering; M. Schuhmacher, Rovira i Virgili University / Chemical Engineering
Most of the current practices in the health risk assessment of exposures to chemicals and heavy metals are based on interspecies correlations and semi-quantitative approaches. However, humans are simultaneously exposed to multiple xenobiotic chemicals (such as pharmaceuticals, metals, pesticides, volatile and semi-volatile organic compounds, etc.) that potentially possess a number of similarity or different toxic effects. If more than one chemical enters the body, a potential arise for interactions among chemicals, their metabolites, and the biological molecules/systems. Consequently, the authorities are challenged to consider that this “chemical cocktail” or “total chemical load” does not produce unforeseen health effects. Combined action and interactions between chemicals, at high doses have been known for many years in the field of pharmacology. However, these in-situ experiences are not directly useful for predicting toxic effects of mixtures of environmental chemicals because the exposure levels of the general human population are relatively low and interactions occurring at high doses may not be representative for low-dose exposures. In case of toxic metals which are transported and eliminated through many common cellular mechanisms by “molecular mimicry” because of their similarities to essential metals, therefore, there exist toxicokinetic and toxicodynamic interactions among toxic and essential metal analogs. Metal analogs should thus be considered highly correlated for exposed individuals, with susceptibilities resulting in differential effects of multiple metals. In this study, we analyse the toxicokinetic and toxicodynamic of Cd, Hg and Pb using generalized whole body physiologically-based pharmacokinetic (PBPK) models for a case study in Spain. Physiological variability in the population is considered across the model by linking with biological databases that provide physiological values for a majority of the tissues groups. Interaction effects between the study heavy metals mixtures are incorporated. Model has been validated with data from previous studies (1998-2007) of human exposure to metals in autopsy tissues of individuals living near a waste incinerator.

Effect Modelling of environmental systems - extrapolation and prediction of species response (P)

TH128

The use of dynamic population-level predictions in PERA: challenges and opportunities
C. Rendal, Unilever / Safety and Environmental Assurance Centre; O.R. Price, Unilever / Colworth Science Park; B. Goussen, University of York / Department of Environment; R. Ashauer, University of York / Environment Population-level models are increasingly recognised as potentially powerful tools in environmental risk assessment. However, the practical application of dynamic population predictions for decision making is not straightforward in everyday risk assessment of down-the-drain chemicals. Probabilistic environmental risk assessment (PERA) has been suggested as a way to make uncertainty more explicit and to account for spatial and temporal variability. The outcome of a PERA is typically a measure of expected risk with an associated uncertainty interval. We propose a conceptual framework that explores how probabilistic approaches can be applied in population models to incorporate more ecological relevance into risk assessments while keeping both uncertainty and variability explicit and transparent. One of the key challenges is understanding the protection goals for ecological scenarios exposed to anthropogenic stressors. For instance, systems that are already impaired by high volume emissions of untreated wastewater may require modified protection goals (e.g. protection of microbial purification processes and recovery of food web structure and diversity). For higher organisms, the protection goals must be reflected by a defined set of endpoint metrics that can quantify changes in population-level dynamics. These metrics must be carefully selected based on both the specific scenario and protection goal, as different options will lead to very different interpretations of effect. Finally we discuss the importance of making the relation between the willingness to accept risk and the severity of the effect explicit to facilitate decision-making. We consider these discussions a necessary first step in bringing the full potential of population-level models into risk assessment of down-the-drain chemicals.

TH129

Interspecies QAAR for pharmaceuticals
A. Sangion, DIISTA; S. Cassani, P. Gramatica, University of Insubria / DIISTA
Due to their extensive and progressive use in human and veterinary medicine, pharmaceuticals have been reported to be ubiquitously in surface and waste waters and are widely considered emerging environmental contaminants. Moreover, pharmaceuticals are specifically designed to be biologically active and their presence in the environment may be of serious concern for the wildlife. Even though a lot is known about pharmaceuticals’ human toxicity, there is a lack of knowledge about their potential environmental hazard and ecotoxicology. In-silico approaches, like those based on quantitative structure–activity relationships (QSARs), are valuable tools to maximize the information contained in existing experimental data; in particular, quantitative activity–activity relationships (QARs) are often used to find correlations and to predict missing information extrapolating from one species to another. In this study we present different interspecies toxicity models between *Daphnia magna* and *Pimephales promelas*, between *Daphnia magna* and *Oncorhynchus mykiss* and between the two species of fish *Pimephales promelas* and *Oncorhynchus mykiss*, for the prediction of acute toxicity of many pharmaceuticals chemicals. In addition to the pure correlation QAARs, we also propose models based on species response and a selected molecular descriptor by the all-subset procedure included in QSARINS software, starting from a matrix of hundreds calculated OD-2D descriptors by PaDEL-Descriptor software. All models presented are stable, robust and validated (R2 > 0.75 and Qloo >0.70) and can be used to extrapolate acute toxicity from and to different trophic levels. Particular attention was given to the structural applicability domain (AD) of the proposed models. We also analyzed the different correlations between the species, highlighting which ones were more correlated and which descriptors have more influence on them. Finally we applied the developed models to predict acute toxicity where data, for at least a species, were present and we compared the predictions derived from different models for the same chemical in order to propose consensus models to extrapolate information from crustacean to fish level.

TH30

Food web models as a tool for ecological scenario analysis
K.P. Väänänen, Ghent University / GhentToxLab; F. De Laender, Université de Mons / ASR; L. Verachtert, Laboratory of Environmental Ecosystem Ecology, C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology GhentToxLab unit
One of the main challenges when evaluating the risk of a chemical for the environment is how to incorporate the large variation between and within ecological systems. A possible approach to deal with this diversity in biotic and abiotic conditions is to perform a set of possible scenario scenarios. Standard scenarios cover a wide set of abiotic conditions e.g. spatial dimensions, hydrodynamics, physicochemical properties of chemicals and emission characteristics. Biological attributes also need to be accounted for e.g. the
degree of isolation or the presence of a predator will contribute to the vulnerability of populations and food webs. It is therefore important to assess the impact of both abiotic and biotic factors on the ecological effects of a chemical. Here, a spatially-explicit food web model was applied to assess the impact of three biotic attributes in ecological scenarios: degree of isolation, food web configuration and life cycle properties (e.g. generation time). The food web model was based on the DEB (Dynamic Energy Budget) Theory. In this DEBkiss model, the food web was described by a DEBkiss individual based model. With this approach, the number of parameters per species was limited, allowing for easy manipulation of the life cycle properties. Different food web configurations were tested by manipulating the number of competing species and predators. The degree of isolation was changed by altering the spatial configuration of individual populations and the migration potential of a species. Using the food web model, we were able to quantify the impact of biotic variables on the outcome of a chemical stress event. We evaluated the relative contribution of each biotic attribute and assessed their importance for ecological risk assessments.

**TH131**

**Effect of metals in estuarine organisms: modelling toxicity in physiologically changing systems**

A. de Souza Machado, Leibniz Institute of Freshwater Ecology and Inland Fisheries / Ecophyiology and Aquaculture; W. Klosa, Leibniz Inst. of Freshwater Ecology & Inland Fish.; C. Zarfl, University of Osuna / Center for Applied Geosciences

In contrast to freshwater, where organisms are hyporiparian, saltwater environments are characterized by a high diversity of physiologically diverse species, such as marine estuarine invertebrates. Marine estuarine invertebrates can hypo/hyper-regulate or even conform to ionic and osmotic pressures of surrounding waters. In estuaries, the frequent salinity changes may shift in ion-osmoregulation strategy critical for survival. Modelling toxicity in estuarine species is related to changing physiology as a major challenge in metal ecotoxicology. Changes in membrane transporters and their composition and individual metal affinity determined by salinity-driven metallothionein adaptations affect contaminant uptake and organism sensitivity or susceptibility. Such enantioselectivity processes mask the effects of metal causing toxicity. Also, most knowledge regarding metal toxicity to aquatic organisms has been established in freshwater, and suggested that the common mechanisms of toxicity are related to ion and osmotic disturbances driven by ion gradients between organism’s plasma and environmental water. However, the lack of models accounting for the enantioselective processes restricts our ability to explain and forecast toxicity in saline gradients. Thus, this study presents a mechanistic model to explain metal toxicity to aquatic organisms due to changing salinity. We propose a conceptual model able to incorporate published results of Cu and Cd effects on estuarine animals. The toxicity in this model is explained by variation in internal ion concentrations, assuming failure in ion-regulation as the main mechanism of action. In turn, internal ion content is represented by parameterization of water chemistry, metal exposure, and enantioselectivity outcomes. The implication of this model is that water chemistry explains most of the metal toxicity in freshwater systems, where an ion-osmoregulation strategy is possible and small changes in water chemistry cause major changes in metabolism. However, the organism’s physiology is dominating the toxicity process, while enantioselectivity is important at intermediate salinities. This mechanistic model is able to integrate conflicting published results where metal toxicity is claimed to be dependent on water chemistry or physiology. Ultimately, it also points out that more metal toxicity mechanisms are to be discovered, especially for exposures occurring at high salinities.

**TH132**

**A standardized approach to identify worst-case FOCUS surface water exposure profiles in aquatic pulsed exposure events**

O. Körner, ADAMA Deutschland GmbH / EU Registration; D. Nickisch, Environmental Fate Modeling

The outcome of Tier 1 risk assessment (RA) for plant protection products (PPP) frequently calls for the use of higher tier approaches to provide an acceptable risk to aquatic organisms. In this context, laboratory pulsed exposure experiments can be used to test for the effects of varying exposure concentrations as it is often the situation in the field. However, due to the highly challenging and often based on subjective expert judgements, TK/TD models are a useful tool that accounts for time variable exposure concentrations and predicts toxicity on organisms. They allow extrapolating toxic effects beyond test conditions while considering real time carry-over toxicity. Hence, TK/TD models provide one means to determine worst-case exposure scenarios in a more straightforward and easy way. However, we hereby use the TK/TD model GUTS to determine the exposure scenario with the strongest impact on organisms. We generate exposure profiles from the different representative EU surface water scenarios defined in the FOCUS-SW models. These models consider various entry pathways of PPPs into the aquatic environment, mainly spray-drift, run-off and drainage. Contrary to the first two pathways for which mitigation measures are available, entry via drainage cannot be reduced and represents the most frequent case for conducting higher tier RA. Besides, entry via drainage can vary considerably in comparison to drift or run-off, leading to more complex exposure profiles. Thus, we select our test substance and application pattern in a way that drainage is the main entry pathway to aquatic systems. The GUTS model is calibrated using survival data from laboratory tests. Using the generated predicted exposure patterns from FOCUS-SW models as input, we run GUTS simulations over the entire time period defined in the FOCUS models and predict survival. The comparison of the predicted organisms’ responses in the different simulated exposure scenarios allows the identification of the worst-case exposure scenario and subsequently, the selection of the relevant exposure profile with which to test in the field. This new test method may significantly improve our understanding of the link between exposure and effects and highlight the importance of TK/TD models in higher tier RA schemes.

**TH133**

**EE2 impacts on zebrafish population dynamics extrapolated from organism level endpoints using a DEB-IBM model**

R. Beaudouin, INERIS / Models for Ecotoxicology and Toxicology METO; J. Devillers, CTIS; F. Brion, INERIS / Ecotoxicology Unit; A.R. Pery, INERIS / TOXICOLOGY AND ECOTOXICOLOGY MODELING UNIT

Data used to estimate the likelihood of adverse ecological effects typically include responses of survival, growth, or reproduction of individuals measured after a specific exposure duration and concentration. The purpose of these experiments is to determine the dose-response relationships integrated in our population model. In addition, at least one experiment was conducted at the population level with this compound [1] and could be used to test the predictions of our model. Two different cases were tested: (i) the exposure scenario conducted by Kidd et al. [1] and (ii) a discontinue exposure at different concentrations environmentally realistic. The exposure periods of the second scenario were very short and can be used to define the dose-response relationships integrated in our population model. In addition, at least one experiment was conducted at the population level with this compound [1] and could be used to test the predictions of our model. Two different cases were tested: (i) the exposure scenario conducted by Kidd et al. [1] (ii) one experiment at the population level endpoints. Our model provided predictions comparable to the observations reported by Kidd et al. [1] notwithstanding the ecophysiology differences between the two species used. [1] Kidd KA, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, Flick RW, 2007. Collapse of a fish population after exposure to a synthetic estrogen. Proc Nat Acad Sci USA 104:8897-8901.

**TH134**

**A generic approach to modelling mayfly population dynamics in the field**

L. M. Wagenführ, Tu. Strauss, Research Institute gaia / Research Institute Gaia; S. Classen, gaiac Institute for Environmental Research RWTH Aachen; T.G. Preuss, BioScopScience / E.M. Ritter, R. Aachen University / Institute for Environmental Research; A. Gers, RWTH Aachen University / Department of Environmental Social and Spatial Change

In scientific and regulatory communities, individual-based models are gaining importance as a tool to bring more ecological realism and more informed effect predictions into the environmental risk assessment of chemicals. In particular, standard model designs are thought to facilitate the acceptance and usage of mechanistic effect models. Therefore, we tested a generic individual-based model for its applicability to Cloeon dipterus an ephemeropteran species common in Europe. Ephemeroptera in general are considered a representative part of secondary production. Hence, the Ephemeroptera larvae have specific biosphere requirements and are therefore used for the characterization of water bodies. The larvae are also known to be sensitive to pollution, but due to their life cycle, chronic toxicity tests with mayfly larvae are complex and costly. In the model, the lifecycle of each C. dipterus individual is described based on equations provided by the dynamic energy budget (DEB) theory. DEB models describe key life history processes such as growth, maturation and reproduction as functions of the energy gained from food assimilation. Similar to real life, modelled population dynamics emerge from individual life histories and interactions of individuals with their environment. Model parameterisation was largely based on literature data; however, a few data gaps have been filled by additional experiments. The literature review also revealed that the standard DEB model had to be adapted to match the ephemeropteran life history strategy. For model testing, the simulation outcomes were compared to data derived from mesocosm studies. In model applications, it was investigated to what extent environmental factors such as temperature, resource availability and predation may trigger seasonal emergence patterns of mayflies.
Spatially-explicit population modelling as a refinement tool for soil invertebrates risk assessment

M. Meli, J. Ludwigs, Rifcon GmbH

Ecological effect models can be an important instrument to add ecological relevance to terrestrial risk assessments. Current refinement options include ex situ bioassays, Terrestrial Ecosystem Models and field surveys, share some limitations, such as the difficulty to link observed effects to a specific toxic component in the soil, to find proper reference sites or soil. Ecological effect models mechanistically link effects at individual level to impacts at the population level, and allow comparison of different contaminated scenarios to a control situation. Unlike in field survey, ecological models allow for an easier interpretation of the results, distinguishing between effects of the different factors implemented, and are therefore a valuable refinement tool for soil risk assessments. We developed a spatially explicit individual-based model of the collembolean Folsomia candida, designed specifically for conducting population-level risk assessments of pesticides. The purpose of the model is to realistically reproduce population dynamics of F. candida with special focus on the dynamic spatial distribution of individuals and on density dependent population regulation. Model rules and parameters are based on data from the literature. For toxicity, data from standard laboratory tests (survival and reproduction) are used; for exposure soil load, application date and DT50 are required. The model has been parameterized and evaluated using Pattern Oriented Modelling: here models are required to simultaneously reproduce a diverse set of patterns. Landscapes used in the simulations represent part of an agricultural field. Environmental variability is iteratively deduced to the mathematical and statistical distribution of food resources in such landscape. Temperature is implicitly taken into account because it affects the values of several life-history parameters. Temperature profiles are derived from the meteorological data used in the FOCUS GW models. Soil moisture is also implicitly represented in the model and it affects the availability of food resource. For model output population density over time is recorded. Therefore, it is possible to assess reductions of population size caused by exposure to toxic compounds or recovery. Model outputs are thus directly linkable to specific protection goals established for soil invertebrates. Results based on a hypothetical substance, and insights gained from using the model for risk assessment submissions will be presented.

TH136

MOSAIC: a web-interface with modelling and statistical tools for ecotoxicology

S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionary Biology; P. Veber, Université Claude Bernard Lyon 1 / Laboratoire de Biométrie et Biologie Evolutive; M. Delignette-Muller, VetAgro Sup / Laboratory of Biometry and Evolutionary Biology.

In ecotoxicology, bioassays are classically conducted according to standard protocols to measure acute or chronic effects of potentially toxic substances on reproduction, growth and/or survival of living animals. MOSAIC, standing for "Modelling and Statistical tools for ecotoxicology", is a new user-friendly web interface dedicated to the mathematical and statistical distribution of food resources in such landscape. Its simple use makes MOSAIC a turnkey decision-making tool for ecotoxicologists and regulators. Without wasting time on extensive mathematical and statistical technicalities, users are given advanced and innovative methods for a valuable quantitative environmental risk assessment. MOSAIC is available at http://phyl.univ-lyon1.fr/software/mosaic/. Today, MOSAIC offers two operational modes: (i) MOSAIC_bioassay, a webtool dedicated to the statistical evaluation of standard or semi-standard (SSD) approach aiming at defining safe levels for toxic compounds in an ecosystem through the calculation of the so-called hazardous concentration for % of the species (HCp), even when the toxicity values are censored [1, 2]; (ii) MOSAIC_repro, which provides users with a (complete) statistical analysis of bioassay reproduction data simultaneously accounting for mortality all along the bioassay. For that purpose, concentration-effect models are fitted within a Bayesian framework. This poster will be organized as a user guide of MOSAIC, with a succession of screenshots illustrating how to analyse data in two simple and user-friendly steps. Details will be given on the different graphics provided by MOSAIC, but also on the way results maybe saved as they are personalized. MOSAIC, but also on the way results are used.
performed. Essentially the test inquires whether, knowing the autocorrelation of one isolated variable, the error yielded in the forecast verification can be improved by taking in account the case of cross correlation with another variable. The work presents the suitability of wavelet methods for the interpretation of river metabolism. We have obtained the Granger tests in the spectral analysis of metabolism several time series (from up to 16 years long) from several Ebro river subcatchments. Different variables and patterns are being observed in each case. These patterns have been associated both with climatic and anthropogenic factors. Our results also show that wavelet methods could be a useful tool for territorial organizations to obtain integrating environmental, economic and social aspects to make decisions in order to manage the basins.

TH140
Species Sensitivity Distributions for Non-Target Plant Studies
J.W. Green, DuPont / Applied Statistics Group
Species Sensitivity Distributions (SSDs) are increasingly requested by regulatory authorities, especially in the EU, as part of product registration submissions. There is some inconsistency among country regulators as to requirements and acceptable methods of fitting and interpreting SSDs, specifically estimating the concentration hazardous to no more than 5% of exposed species (HC5). A comparison will be made of the median HC5 and lower bound HC5 calculations. Specifically, the variability of the lower bound HC5 will be shown to greatly exceed that of the median HC5 estimate. It has become common in some EU registrations to reject species data for SSD purposes if the data do not conform to the log-normal distribution. There is no assessment basis for this requirement and it is shown that the log-normal is not always appropriate and the consequence of mis-specifying the distribution can be a seriously misleading HC5 value. The EUFRAM work of several years ago recommended capturing uncertainty as well as variability, but some software packages ignore this and report unrealistically short confidence intervals. Datasets used for SSDs often contain censored values (e.g., EC50), the most common regulatory authority assumption. There is no scientific basis for such action and there have long been known scientifically correct ways to include censored values in fitting a distribution. That approach will be described and has been implemented in proprietary software, but only recently has a software package been widely available that implements this approach. The package, MOSVAR, and several other software packages for fitting a SSD and estimating HC5, such as ETX, BurriLOZ, SAS, and SSD Master will be discussed and compared. Finally, some attention will be given to the number of non-target plant species needed for SSD fitting. While the focus of this presentation is on non-target plant studies, the issues presented apply to all SSD work.

TH141
Theoretically exploring direct and indirect chemical effects across ecological and exposure scenarios using mechanistic fate and effects modelling
F. De Laender, Université de Namur ASBL / Laboratory of Environmental Ecosystem Ecology; M. Morselli, University of Insubria / Department of Science and High Technology; h. baveczo, Environmental Risk Assessment Team; P. van den Bulcke, Data Management Group b Alterra; A. Di Guardo, University of Insubria / Department of Science and High Technology
Predicting ecosystem response to chemicals is a complex problem in ecotoxicology and a challenge for risk assessors. The variables potentially influencing chemical fate and exposure define the exposure scenario while the variables determining effects are related to the ecosystem as a whole. In the absence of any empirical data, the objective of this paper is to present simulations by a fugacity-based fate model and a differential equation-based ecosystem model to theoretically explore how direct and indirect effects on invertebrate shallow pond communities vary with changing ecological and exposure scenarios. These simulations suggest that direct and indirect effects are larger in mesotrophic systems than in oligotrophic systems. In both trophic states, interaction strength (quantified using grazing rates) was suggested a more important driver for the size and recovery from direct and indirect effects than immigration rate. In general, weak interactions led to smaller direct and indirect effects. For chemicals targeting mesozooplankton only, indirect effects were common in (simple) food chains but rare in complex food webs. For chemicals targeting microzooplankton, the dominant zooplankton group in the modelled community, indirect effects occurred both in food-chains and food-webs. We conclude that the choice of the ecological and exposure scenarios in ecotoxicological modelling efforts needs to be justified because of its influence on the prevalence and magnitude of the predicted effects. Overall, more work needs to be done to empirically test the theoretical expectations formulated here.

TH142
Overview of NOAA Deepwater Horizon Natural Resource Damage Assessment toxicity testing program
In support of the Deepwater Horizon (DWH) Natural Resource Damage Assessment, NOAA has conducted over 500 bioassays and related chemical characterizations to determine the toxicity of DWH oil to native species in the Gulf of Mexico. The study matrix for this effort included over 25 species of fish and invertebrates; life stages ranging from gametes to adults; a suite of lethal and sublethal endpoints; four different DWH oils and dispersants; and exposure routes, including water accommodated fractions, surface slicks, and sediments. These studies were conducted by over a dozen collaborating university, government, and private laboratories. Analyses of these data are ongoing but we will discuss effects levels for a range of species, exposure chemistry, testing methods, and our meta-analysis of this dataset.

TH143
Toxicity of organic compounds toward Saccharomyces cerevisiae - An In Vitro Bioassay to Assess the Mutagenic Potency of Ectotherms
S. Schweiger, Helmholtz Centre for Environmental Research GmbH - UFZ / Ecological chemistry; G. Schuurmann, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry
The European regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) concerns the production and use of chemicals and their potential influence on human health and the environment. A major aim for REACH is the reduction of animal testing. In order to assess risk potentials of chemicals, the concept of integrated test strategies was developed, which combines data from alternative test methods, like in vitro, in chemico and in silico studies. In the aquatic ecotoxicology, the narcosis model describes that every chemical has a toxicity corresponding to the hydrophobicity. Substances with a lower EC50 as compared to the corresponding baseline narcosis also have a higher toxicity, which can be specific or reactive toxic. The latter of which can be quantified by the toxicity enhancement T Smooth reactive toxic chemicals can interact with biomolecules in cells where they build mostly irreversible covalent bondings, leading to enhanced toxicity, mutagenicity or skin sensitization. In this study a differential growth-inhibition bioassay with two yeast strains was used to evaluate the toxic potential of different organic substances. A strain without the gene Rad54, besides the wildtype of Saccharomyces cerevisiae, was used to determine the genotoxicity of substances. Rad54 is involved in the recombinational repair of double-strand breaks in DNA, that is why it can not repair DNA-doublestrand breaks and thus it is more sensitive to DNA-reactive substances. In our study the concentration of a substance which causes 50% reduction of growth of the yeast cells was determined (effective concentration at 50%, EC50). The ratio of the EC50 of both strains can show that the test substance is genotoxic or rather destroys DNA-double strands (TR-value, toxic ratio). In order to determine the narcosis level of S. cerevisiae a narcosis baseline for both strains for substances from a log Kow of 1.36 to 5.13 were analysed. Two different substance classes, acrylates and sulfonates, were evaluated in a differential bioassay to test the potential of the assay for marine and freshwater substances. All EC50-values were corrected for sorption and evaporation to quantify the freely dissolved fraction in solution. Acknowledgement - The authors thank the SAB (Sächsische Aufbaubank) for financial support (Contract-No: 100119621).

TH144
Dynamic modeling of physiological processes related to energy management in Gammarus fossarum
C. Lopes, Université Lyon 1, UMR CNRS 5558 / Laboratory of Biometry and Evolutionary Biology; F. Mounier, Lyon University 1 / Laboratory of Biometry and Evolutionary Biology; S. Charles, Lyon University 1 / Laboratory of Biometry and Evolutionary Biology; a. chaumot, O. Geffard, Istrea / UR MALY Laboratory of Ecological and Evolutionary Biology
Approaches for the assessment of environmental impacts of contamination rely on toxic effects observed mainly at the individual level. These effects are then extrapolated to higher levels of biological organization (population, community and ecosystem) that constitute the protection goals of environmental regulations (REACH, WFD). Individual performance is governed by the allocation of energy to the physiological processes. DEB theory (Dynamic Energy Budget) formalizes the allocation of energy to key physiological functions (growth, maintenance and reproduction). However, until now, these models assume a permanent energy allocation to the reproduction during the lifespan of an organism, while it is generally a discrete process driven by physiological constraints. This is particularly true for crustaceans for which reproduction is synchronized with the reproduction of a keystone species such as Gammarus fossarum, a keystone species playing an important role in the functioning of European freshwater ecosystems. The aims of our presentation are (1) to present a mechanistic DEB model describing the allocation of energy during a reproductive cycle of G. fossarum females exposed to different temperatures and (2) to illustrate how it can be used under stress conditions. According to the knowledge on G. fossarum reproductive cycle, we schematized the biomass fluxes occurring during a reproductive cycle and formalized them by six differential equations. Considering different temperatures and different sizes of females allowed to consider different feeding rate values for the calibration of energetic processes implied during a reproductive cycle. Parameters were then estimated by Bayesian inference: we obtained precise estimations for the parameters. In particular, we estimated that females allocate 12% [0.11-0.14] of the assimilated energy to reproduction and predicted that 83% of the observations were within the credible intervals. The model was then validated on other data. Finally, we fixed the scheme of energy allocation and compared the model predictions with lower food inputs to what is observed when females are under such food limited conditions.
conditions. We showed that the more the food was restricted, the more the model accurately predicts time-specific lethal effect concentrations for these different freshwater organisms. In addition, the model is able to capture the observed size-specific variation of nearly two orders of magnitude in empirical LC₅₀ values.

IV. Conclusion

In conclusion, we have developed a novel TKTD model for freshwater organisms that is capable of predicting acute Ag toxicity based on TKTD models and our comprehensive database of empirical Ag toxicity data. This model provides a valuable tool for risk assessors and researchers in the field of aquatic toxicology.
TH150  Modelling tissue-specific accumulation of metals in parasite–fish systems Y.T. Le, Radboud University Nijmegen / Environmental Science; M. Nachev, D. Grabner, University of Duisburg-Essen / Aquatic Ecology; J.A. Hendriks, Radboud University Nijmegen / Department of Environmental Sciences; B. Sures, University of Duisburg-Essen / Aquatic Ecology

In the environment, fish are affected by exposure to metals and infection with parasites. Moreover, each of these two factors may affect the response of fish to the other. Metal exposure may reduce the resistance of fish to parasites while parasites may have effects on metal accumulation and metallothionein induction in the fish host. A number of parasites have been reported to have remarkably high capacity of accumulating metals. Therefore, inclusion of parasites might provide more reliable assessment and more accurate interpretation of biomonitoring data. Currently, the partitioning of metals between parasites and their fish host is usually expressed by an empirical ratio of the metal concentration in parasites to that in the host. This factor does not provide a mechanistic understanding of underlying processes determining metal accumulation in parasite–fish systems. Effects of parasites on metal accumulation in the fish host cannot be explained by the uptake by the parasites only. Parasites might influence metal uptake and elimination in the fish host. In addition, parasitism may lead to changes in the subcellular partitioning in cytosolic and particulate fractions, therefore affecting the internal distribution of metals between fish organs. We developed a multiple-compartment model for predicting tissue-specific accumulation of $^{210}$Pb in chub Squalius cephalus uninfected and infected with the acanthocephalan Pompomphorhynchus luevi. The model covers in seven compartments, namely blood, storage, gills, kidney, liver, intestine, and gallbladder. External and internal exchange in the model was related to fish weight, while various effects of the parasite on metal toxicokinetics and tissue-blood partitioning in the chub were accounted for in the model. In the model for infected chub, it was assumed that while taking up essential fatty acids, parasites absorb organo-metallic complexes formed between metals and bile acids excreted to the intestine. The model predictions show high tissue-blood partitioning in liver, attributed to the role of this organ as a detoxifying compartment. Assuming different effects of parasites on the uptake (enhancement) and elimination (alleviation) in the host, model predictions indicate increasing internal exchange between intestine, where the parasite resides, and other organs. Generally, the modelled concentrations of $^{210}$Pb were within one order of magnitude of the measured, for both infected and uninfected chub.

Addressing data collection and computational challenges in LCI (P)

TH151  The new Reviewer Registry of the European Platform on LCA S. Fazio, Institute for Environment and Sustainability; D. Pennington, European Commission

To support EU environmental policies based on Life Cycle approach, the European Commission hosts the "European Platform on Life Cycle Assessment (EPLCA)". The Platform aims at providing coherent and quality-assured life cycle data, methods, and studies as well as information about developers and suppliers of LCA dedicated software and databases (DB), and service providers. The LCA Resources Directory (RD), aims to offer a sharing platform for information related to software and database developers/service providers, and service providers within the LCA community, there is also a repository for studies and documents with specific templates allowing to upload studies on e.g. Product and Organisation Environmental Footprint, Ecodesign, EPD and related Category Rules/Criteria. Recently a new tool has been added to the Resource Directory, i.e. the Reviewer Registry, would provide a list of qualified (i.e. with different levels of compliance for Life Cycle based methods) reviewers from different countries/sectors. The Reviewer Registry is a tool designed to provide information about reviewers concerning their expertise, knowledge and other relevant info. It helps the users to identify potential appropriate reviewers for their LCA studies. Based on the info provided, the tool assigns a score (now based on International reference Life Cycle Data system (ILCD) handbook, and Environmental Footprint (EF) requirements). INFO PROVIDED: languages spoken, countries covered, Career info, education list of employers, years of experience, number of reviews, Accreditation as third party reviewer, references, Review practice review practice chair, Review practice trainer, sectors of work experience and years, etc. a searching tool has been also provided, the tool assigns a score (now based on International reference Life Cycle Data system (ILCD) handbook, and Environmental Footprint (EF) requirements). The mechatronic sector is a strategic sector from the economic and environmental point of view, mainly due to the high content of strategic raw materials used such as (precious) metals, and also on the high production rate, short service life and, in turn, to the high waste flows they generate. The end-of-life (EoL) management of mechatronic products and the assessment of associated environmental impacts play thus a relevant role in the roadmap to resources efficient Europe, and several methodological/practical approaches have been developed. Among them, the Product Environmental Footprint (PEF) methodology (1), currently being tested through pilot projects in different sectors, proposes a new EoL approach, which has raised the criticisms of many stakeholders in different sectors. This paper discusses main challenges and insights from the PEF EoL application to the case study of a domestic cooker hood, and attempts to answer the following questions: what kind of PEF EoL approach is delivered by the PEF formula proposed by the PEF to the EoL scenario of cooker hood component, namely the aluminium filter component and, results have been compared with results arising by the application of one of the most used approaches in the metal sector, i.e. the “avoided impact approach”. Environmental impacts delivered by PEF EoL approach is higher than environmental impacts delivered by the avoided impact approach. Main methodological/practical aspects arising from PEF EoL approach application to the studied product concern: 1) the definition of the recycled content; 2) the identification of disposal process to which recycled content of material has been diverted; 3) the definition of recycling fraction.

TH153  Consistent allocation for co-production and recycling in LCA - A critical review of guidelines D.L. Schrijvers, The Life Cycle Group CyVi; G. Sonnemann, University of Bordeaux 1 / Life Cycle Group CyVi; Y.T. Le, Institute for Environment and Resources, Bordeaux 1 / The Life Cycle Group CyVi

Recycling and co-product allocation have always been points of debate in Life Cycle Assessment (LCA). Several guidelines have proposed allocation procedures, but these recommendations are often divergent. We have reviewed allocation procedures of co-products and in recycling and recovery situations using a consistent and systematic approach, considering all material types and allowing for different LCA goals. The allocation methods that are most often used – allocation, the cut-off approach and substitution, which comprises the end-of-life recycling method, substitution for the recycled content and the 50/50 method – are mathematically compared to show how multiple recycling loops are considered and how the methods influence the LCA results. Based on the underlying values and assumptions of the methods, a systematic framework is developed. A strict separation between attributional and consequential LCA related to different LCA goals is proposed for discussion, following the UNEP/SETAC Shonan declaration of the consistency of attributional LCA. This framework is used as basis for the critical review of recent guidelines regarding the consistency of their allocation procedures. Only the ILCD Handbook proposes a consistent approach in two of the four scenarios that are described. Often different approaches are recommended for co-products and recycled materials, although the boundary between these flows is not always clear. Many guidelines do not recognize the existence of different LCA goals, therefore elements of attributional and consequential LCA are often mixed, e.g. by recommending both the cut-off approach and the end-of-life recycling method. The market situation of the recycled material is not always taken into account, e.g. in the material balance of 50/50 method. Based on ISO/TS 14007 we propose a new substitution formula, replacing the arbitrary ratio of 50% by the market price ratio A. The main obstacles for consistent modelling are the different approaches for co-production and recycling and disregarding of different LCA goals and recycled material markets. We recommend to include material specific guidance in PCR documents on the determination of the avoided process, market price ratios, value-correction factors and relevant allocation criteria. The new substitution formula will be tested on case studies for organic chemistry and metals to show its applicability to credit for the recycling activity that actually leads to environmental benefits.

TH154  Consistent allocation in LCA - Can avoided impacts be modelled in attributional LCA? D.L. Schrijvers, The Life Cycle Group CyVi; G. Sonnemann, University of Bordeaux 1 / The Life Cycle Group CyVi

LCA studies have recently been divided into attributional and consequential LCA. Each approach serves a different goal definition and allocation methods that are most often used – allocation, the cut-off approach and substitution, which comprises the end-of-life recycling method, substitution for the recycled content and the 50/50 method – are mathematically compared to show how multiple recycling loops are considered and how the methods influence the LCA results. Based on the underlying values and assumptions of the methods, a systematic framework is developed. A strict separation between attributional and consequential LCA related to different LCA goals is proposed for discussion, following the UNEP/SETAC Shonan declaration of the consistency of attributional LCA. This framework is used as basis for the critical review of recent guidelines regarding the consistency of their allocation procedures. Only the ILCD Handbook proposes a consistent approach in two of the four scenarios that are described. Often different approaches are recommended for co-products and recycled materials, although the boundary between these flows is not always clear. Many guidelines do not recognize the existence of different LCA goals, therefore elements of attributional and consequential LCA are often mixed, e.g. by recommending both the cut-off approach and the end-of-life recycling method. The market situation of the recycled material is not always taken into account, e.g. in the material balance of 50/50 method. Based on ISO/TS 14007 we propose a new substitution formula, replacing the arbitrary ratio of 50% by the market price ratio A. The main obstacles for consistent modelling are the different approaches for co-production and recycling and disregarding of different LCA goals and recycled material markets. We recommend to include material specific guidance in PCR documents on the determination of the avoided process, market price ratios, value-correction factors and relevant allocation criteria. The new substitution formula will be tested on case studies for organic chemistry and metals to show its applicability to credit for the recycling activity that actually leads to environmental benefits.
system, often referred to as substitution, the avoided burden approach or system expansion. Although both approaches give the same results, both perspectives are interesting topics for a discussion. Indicating one conventional process – or even a production mix – as the default production process for a certain (co-)product, i.e. in the proxy perspective, might disregard the potential of the multifunctional processes to be an alternative production process that even could contribute to the production mix for which its specific inventory could be determined. The definition of attributional LCA is relevant for the subsystem perspective: “Describing the inputs and their associated emissions attributed to the delivery of a specified amount of the product functional unit” (PAS 2050) could leave room for applying substitution. However, if “the attributional approach attempts to provide information on what portion of global burdens can be associated with a product (and its life cycle)” (UNESETAC, 2011), substitution might result into losing impacts between product systems, as Heijungs already argued in his PhD thesis (1997). As both definitions can facilitate relevant LCA studies, e.g. for comparing products, we recommend to clearly distinguish between LCA purposes and these definitions, for consistent application of allocation procedures.

TH155 Assistox: A data management tool for the calculation of toxicity characterization factors

G. Rodriguez Garcia, Helmholtz Institute Ulm / Systems III; M. Weil, Karlsruhe Institute of Technology KIT / Institute for Technology Assessment and Systems Analysis ITAS

In the Life Cycle Assessment (LCA) studies, toxicity impact assessment categories are often not included or discussed in depth. Two possible reasons for that are: a) the perception that they are a black box and less reliable than other impact categories, and b) the lack of characterization factors (CF) for potentially key pollutants. We have developed Assistox, a tool to facilitate the calculation of CF with USEtox impact assessment methodology. It consists of two main components: s) a detailed procedure that guides LCA practitioners through data made available by the European REACH regulation and other freely-available databases, and b) an Excel spreadsheet that transforms collected information into ready-to-export data for the aforementioned methodologies. The ongoing validation process suggests that the differences between the CFs calculated using Assistox and those already available for USEtox are not statistically significant for Freshwater Ecotoxicity and for terrestrial Toxicity. The results indicate it would be possible to obtain compatible CF for new substances. It is our opinion that tools like Assistox can make toxicity impact categories more accessible, and will foster the use of these categories in future LCA studies.

TH156 Results of the ERASM Surfactant Life Cycle and Ecofootprinting (SLE) project: New and updated inventory data for oleochemical and petrochemical surfactants

K. Groenke, Evonik Industries AG / Department ESH; A. Bouvy, Cefic; D. Eggers, PE International AG, E. SLE Task-Force, ERASM

The objective of the SLE project was to update and establish the environmental profile of the major commercial surfactants and their precursors in Europe based on consistent and high quality (background) data. Fifteen companies collaborating within ERASM (www.erasm.org) compiled ‘cradle-to-gate’ life cycle inventories (LCIs) data for state of the art production of a series of common surfactants used in detergents and personal care products. The intention of the environmental profiles is to support the modelling of LCAs in industry as well as in various policy initiatives and provide data for ‘cradle-to-grave’ assessments of consumer products. For each major unit process in the surfactant production at least three companies (‘trios’) were identified, which confidentially provided process data to the project’s independent consultant, hence allowing to produce an aggregated anonymous average. Literature data were used where it was difficult to collect new data within the scope of this project, or in case recent and reliable process data were available. The LCI methodology was compliant to the international LCA standards ISO 14040:2006 and ISO 14044:2006. Two independent external reviewers, focusing primarily on LCA methodology conformity and process technology, supported the project from the beginning through completion. In addition, the data for palm (kernel oil) and coconut oil-based renewable precursors were re-evaluated by a third expert who was a specialist in the matter. The ERASM SLE project has delivered new and updated LCI data for 15 surfactants and 22 precursors in three common LCI exchange formats: ILCD, EcoSpold v.2 and GaBi. The data reported represent an industry-agreed and representative market average for surfactants in European consumer products for the reference year 2011. Similarities and differences with previously published LCI data (1995) are discussed, as well as the novel approach to calculate the greenhouse gas emissions resulting from land use changes in Malaysia and Indonesia associated with the cultivation of renewable precursors.

TH157 National Level LCA of Potable Water Supply System - Key Challenges and Solutions in Data Collection

N. Merov, The Porter School of Environmental Studies; V. Blass, Tel-Aviv University / The Recanati Graduate School of Business Administration

This study generates Israel’s LCI dataset of tap water and reclaimed water. For many years severe water scarcity in Israel has been a driver for the development of advanced water technologies. High quality water supply throughout the entire country has been made possible through a vast and complex water supply system. The contribution of the Israeli advanced water supply system to past and present development of agriculture and industry have been studied in detail. Less known is the associated environmental impact of the Israeli water system, an issue of growing importance as the protection of environment and the reduction of environmental impacts become a central theme in decision making. Our research on the LCI dataset of tap water in Israel covers the entire value chain of the country’s highly complex water supply system. It includes: water extraction and production from a variety of sources such as groundwater, surface water, brackish water and sea water; water treatment; desalination; a national transmission system, which runs throughout the entire country; distribution and tertiary treatment of wastewater for water reclamation. The study comprises data collection for all components of the Israeli water system, resulting in the first national level LCI of tap water. To carry out a national level study there was a need to collect data from a wide array of sources, public and private. The collected data is related to energy and chemical consumption, materials for infrastructure, and transportation of materials. The data collection process introduced major challenges such as: the lack of access to data sources, in particular private data sources, limited availability of old data and non-aggregated data, and incompatibility of data from various sources. Our approach to resolve these challenges included stakeholder engagement and data processing. The study used the ReCiPe method to assess the impacts of the system on climate change, water depletion, freshwater and marine water eutrophication, freshwater and marine aquatic ecotoxicity, and terrestrial and atmospheric acidification.

TH158 Life cycle inventory of a new process employing rice husk ash for the inertization of air pollution control residues coming from MSWI

G. Tomasoni, Università degli Studi di Brescia / Department of Mechanical and Industrial Engineering; C. Moroni, University of Brescia / Department of Mechanical and Industrial Engineering; M. Collotta, University of Brescia; M. Alberti, University of Brescia / Department of Mechanical and Industrial Engineering

In this work the methodology and the main results of the Life Cycle Inventory (LCI) of the Life Cycle Assessment (LCA) of the Cosmos-rice process are presented. Cosmos-rice is a new process for the inertization of air pollution control residues coming from Municipal Solid Waste Incineration (MSWI). The inertization process, which has been developed by the Chemi4Tech laboratory of the University of Brescia, uses as stabilizing agent rice husk ash. The LCI analysis conducted over a new technology bears some problems, due, for example, to the lack of primary data (i.e. data for the specific production of Cosmos rice), which in the present study was provided by the producer to complement secondary data (i.e. data taken from literature and databases) on rice process. The LCIA results were performed with the ReCiPe 2016 method, providing three impact categories: Acidification (both water and terrestrial), Eutrophication (both marine and freshwater), and Climate change. The recent impact categories, influenced by the LCIA approach chosen in the database for crops that are grown in the tropics. The system boundary assessment of the process that involves the production of rice husk ash is presented in the next section. Besides, the use of rice husk ash as a stabilizing agent for air pollution control residues allows to recover and reuse of a material that is currently considered as waste. The study comprises data collection for all components of the LCA, resulting in the first national level LCI of a new process.

TH159 Database selection - be aware of the implications!

M. De Munck, PRe Consultants; T. Pisoni, PRe Consultants / Consultancy OBJECTIVES In LCA software, multiple inventory databases are available that can be used for background processes in an LCA. The way the emissions and resource extractions in the background data are modelled can be of crucial importance for the results of a product life cycle assessment. The question is whether the influence of this modelling approach matches the goal and scope of the LCA study. This poster demonstrates the potential impact of differences in the modelling approach chosen in the database for crops that are grown in the tropics.

To carry out our research we selected a set of products from different sectors, present in multiple databases (≥2 of the following databases: ELCI, ULCI, Agri-footprint, ecoinvent 2.2, ecoinvent 3.1 cut-off), and compared their impact with 2 different methods in SimaPro 8.0.4: ReCiPe Endpoint (H) and IMPACT2002+. RESULTS The following categories showed large differences between databases in several cases: Climate change: we observed important differences due to differences in the input-output balance, especially in case of crops. Natural land transformation: we observed extreme differences due to the modelling approach chosen in the database for crops that are grown in the tropics. Toxicity: we observed important differences in flows of metals to the soil which can be very relevant (e.g. Arsenic). Conclusions We recommend to always be aware of the implications when selecting a database for a modelling approach. The influence can be positive (emission) or negative (take up), as well as differences in the presence or absence of substances and the accompanying quantities. Particulate matter formation: we observed important differences in the level of detail of the inventory data (particle size) and assumptions on ammonia emissions from the soil. It is important to note that differences between databases will not always be visible in the output; this depends on the selected method.

CONCLUSIONS When conducting an LCA with background LCI data, the results can be influenced significantly by assumptions and/or shortcomings of the selected database, particularly for climate change, natural land transformation, toxicity, and particulate matter formation. This study emphasizes the importance of selecting a second method to reveal ‘potentially hidden impacts’. We recommend to check
whether the modelling approach of the background data matches the goal and scope. Future research should include the development of more alternative inventory databases, in order to reveal shortcomings and hidden assumptions of LCI databases and to enable practitioners to select the database that best matches the goal and scope of their LCA.

TH160
Modular, parameterized life cycle inventories of transport of goods in need of atmosphere control
T. Levorg: E. Moreno Ruiz, ecoinvest; G. Bourgault, Ecoinvent Center
Nowadays, when goods are being traded all over the world on an increasing scale, the significance of the freight transport within the overall environmental impacts of products is increasing as well. While some goods do not need specific conditions during their transport, others, such as food, need to be stored and transported while maintaining certain atmosphere (temperature, air flow, etc.). The atmosphere control, of course, requires specific infrastructure and energy for the operation of the infrastructure. This project focuses on creation of datasets, using different sources of information - such as publically available databases - and the latest LCI modelling options available thanks to the use of ecoSpold2 format. The datasets on the undefined unit process level are built so that the modelling is transparent, easily adaptable, well documented and it is possible to demonstrate the differences in the modelling using the three different system models currently offered by the ecoinvest database. The life cycle inventories are built in ecoSpold2 format using variable names, mathematical relations and parameters. All datasets are parameterized by considering the functionality and key parameters of the whole system such as type of goods, refrigerant, distance and duration of transport, etc. The preliminary results, using IPCC 2007, 100a method, demonstrate that the final impact of a product might be increased by around 9% when transport with atmosphere control is included. This corresponds with values published in other studies. This value might vary significantly in specific cases and further research is needed to produce more reliable value for different type of goods.

TH161
Simplified modelling of environmental impacts of foods
F. PERNOLLET, INRA, UMR SAS / UMR SAS; H.M. van der Werf, INRA, UMR SAS / Environnement etAgronomie
Human nutrition strongly contributes to several environmental impacts, resulting in more and more LCA studies on foods and diets. However these studies highlight a lack of data on food products. As a result, these studies make huge simplifications, such as reducing the number of foods considered, using a proxy to model a group of foods, modelling only the stage up to the farm gate or to the retailer instead of the full life cycle, or considering only the climate change impact. These simplifications can strongly affect the results. This study proposes three methods to simplify impact assessment of food products, considering time available and required robustness of results. The first method is based on the fact that, for a food product, most of the impact is often due to the farm stage, for which data are often available or can be estimated relatively easily. The impact of the food product can then be estimated using information on crop coefficients and on energy and land occupation, and results were compared to those of a detailed cradle to plate LCA. The second method improves the first method by adding cooking impacts, and the third method adds transport impacts. The three methods were tested on a French average diet for the impacts climate change, cumulative energy demand (CED), acidification, eutrophication and land occupation and results were compared to those of a detailed cradle to plate LCA of the diet. Except for CED, and relative to the detailed LCA, the three methods yielded a better estimate than a detailed cradle to retailer LCA. Compared to the detailed LCA, the underestimations (in %) obtained with methods 1 and 3 were respectively (24, 60, 10, 9, 8) and (12, 30, 5, 6, 8) for climate change, cumulative energy demand, acidification, eutrophication and land occupation. Methods 1, 2, 3 and the detailed cradle to plate LCA are increasingly time-consuming. Method 1 is adapted for products with a high cradle to farm impact such as dairy and meat products. Method 3 is adapted for fruit, vegetables, pulses, dairy and meat products, and is too imprecise for products with high-impact packaging (can and glass), or for products with a high energy demand during industrial transformation such as coffee, semolina, ultra-high temperature milk. These methods should be tested on more diet types and for more impacts for validation.

TH162
REnCA a case study approach for assessing bioenergy chains on a regional level
S. Okeeffe, Helmholtz centre for environmental research - UFZ / BEN; S. Majer, Deutsche Biomassemessung und dezentrale Energieversorgung GmbH / DREZ; H.M. van der Werf, INRA, UMR SAS / Environnement etAgronomie
For well over a decade, the energy conversion systems have been shifting from globalised scales to more regional and decentralised scales in the form of biomass-to-bioenergy chains. Unlike its fossil counterpart, biomass is scattered in a diffuse manner across a region. Therefore, in order to identify the potential environmental burdens associated with regional bioenergy production, the spatial configurations of bioenergy chains and thus the related regional conditions need to be assessed. The LCA approach for regional bioenergy systems, or REnCA, was applied to the Central Germany region. The REnCA approach expands on traditional life cycle methods, using a "hybrid" life cycle inventory approach, as it involves integrating the results of different modelling approaches, ESRI ArcGIS®, Matlab and LCA tools (GABI 6.0). The combination of these modelling approaches enables the assessment of spatially disaggregated foreground data at the regional level for biomass-to-bioenergy production chains, while also linking to background processes. Background processes, or non-regional processes, in this case refers to all those activities outside the regional scope. In making this separation, the foreground data can then be coupled with other modelling approaches in order to generate a more regionally distributed life cycle inventory.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (P)

TH163
Recent global regulatory developments and data requirements for endocrine disruptor testing and assessment
M. Daft, DR. KNOELL CONSULT GmbH / ICEHESCOM; I. Conrad, DR. KNOELL CONSULT GmbH / Agrochemicals Ecotoxicology
Despite decades of scientific research and extensive discussions and work within regulatory panels, an intended consensus on the assessment of substances with an endocrine disrupting potential, so-called endocrine disruptors, has not yet been reached or is not even conceivable in the near future. 'n Several diverging proposals for an assessment of plant protection products or biocidal products to consider potential endocrine effects are available within the European Union, e.g. by Denmark, UK/Germany or France. Under REACH, substances with endocrine disrupting potential can be considered as substances of very high concern and may be subject to authorization. Whereas the European proposals are mostly aiming at a hazard-based assessment proposing endocrine disrupting properties as cut-off criterion with few exceptions, US EPA follows a different approach and presents a comprehensive two-tiered screening program still to be followed by risk-based assessment in other areas, e.g. Canada, Japan, China or Korea. National programs for the assessment of endocrine disrupting chemicals have been launched or are about to follow other proposals. As scientific criteria for the evaluation of endocrine disrupting properties of a substance are still not yet available, assessment is mostly conducted on a case-by-case basis at the moment. 'n For companies intending or supporting global registrations for their substances, this results in substantial uncertainty regarding data requirements or testing and assessment strategies. This presentation aims to give a global overview on present regulatory proposals, recent regulatory developments and data requirements for the assessment of endocrine disrupting chemicals.

TH164
An Analysis of Control Data from the US Endocrine Disruptor Screening Program List 1 Chemical Screening Battery Results
A. Schapaugh, Monsanto Company; L. McFadden, The Dow Chemical Company / Toxicology Environmental Research and Consulting; J.W. Green, DuPont / Applied Statistics Group; L. Zorilla, Bayer CropScience LP; A.W. Olmstead, Bayer CropScience / Environmental Toxicology and Risk Assessment; C. Borgert, Agrochemicals Ecotoxicology Environmental Research and Consulting; L. McFadden, The Dow Chemical Company / Aquatic Toxicology; H. M. van der Werf, INRA, UMR SAS / Environnement etAgronomie
Endocrine Disruptors: Exposure, Hazard & Risk Assessment

TH165
Despite the widespread occurrence of endocrine disrupting chemicals and the potential for adverse effects such as reproductive and developmental effects, there is still much uncertainty regarding the general health effects of exposure to these chemicals. For companies intending or supporting global registrations for their substances, this results in substantial uncertainty regarding data requirements or testing and assessment strategies. This presentation aims to give a global overview on present regulatory proposals, recent regulatory developments and data requirements for the assessment of endocrine disrupting chemicals.
Thyroid disruption in developing zebrafish (Danio rerio) embryos - effects on gene-expression and morphology

I. A. Baumann, University of Bern / Centre for Fish an Wildlife Health; K. Rehberger, University of Bern / FiWW; H. Segner, University of Bern / Centre for Fish and Wildlife Health

Environmental contaminants with endocrine disrupting properties are not only able to disrupt the reproductive system of fish, but also their thyroid system. Comparably little is known about the developmental, physiological and behavioral consequences of thyroid disruption in fish. At the same time, thyroid hormones play an important role in key physiological processes such as growth, development and reproduction, its environmentally induced disruption can impact a variety of health and fitness-related parameters such as larval survival, metabolism, behavior or fertility. These organism-level changes can then propagate into altered population demographics due to selective mortality. Consequently, there is urgent need for more intensive research on the effects of thyroid disruption in fish. The present study aims to investigate the relation between initiating molecular events of thyroid disruption and morphological changes of the embryonic development of zebrafish (Danio rerio). Two modes of action were studied: interference with thyroid hormone synthesis with the pharmaceutical Propylthiouracil (PTU), and binding to thyroid hormone receptors with the flame retardant Tetrabromobisphenol A. Changes in gene-expression were analyzed with qRT-PCR and visualized with whole mount in situ hybridization. The morphological changes were assessed by means of histologic and morphometric analyses. The results provide initial insight into how chemical interactions with molecular events in the thyroid axis of zebrafish translate into morphological alterations of the developing embryos.
TH170

Synthetic glucocorticoids in the aquatic environment: effects on zebrafish (Danio rerio) embryos

A.O. Hidasi; Eawag - Swiss federal Institute of Aquatic Science and Technology / Environmental Toxicology; K. Joth, Eawag / UTCOX Environmental Toxicology; M. Butler, Eawag - Swiss federal Institute of Aquatic Science and Technology / Environmental Toxicology; K. Schmider, Eawag / Environmental Toxicology.

Synthetic glucocorticoids (GCs) are frequently used in medicine. These compounds were detected in the aquatic environment in the ng/L range. GCs mimic cortisol, the natural stress hormone, by acting through the glucocorticoid receptor (GR) and altering target gene expression. Endogenously, cortisol is produced in fish when the hypothalamic-pituitary-adrenal (HPA) axis is activated. Zebrafish embryos are used to investigate whether exposure to environmentally relevant concentrations of a model GC, clostebol propionate (CP), results in GC-related effects, such as impairment of the stress response and immunosuppression. We avoid using an organic solvent carrier, such as DMSO, due to the experienced solvent effects. We are focusing on nonnolar concentrations, and since CP can be dissolved in the embryo medium up to 1000 nM, no precipitation is expected. Zebrafish embryos are exposed from 1-96 hours post fertilization (hpf). In order to investigate if the effect of CP exposure on the stress response, the cortisol level of non-stressed and stressed embryos is measured by ELISA.

Jung, Seoul National University / Institute of Health and Environment; K. Youn, Seoul National University; A.O. Hidasi, Eawag - Swiss federal Institute of Aquatic Science and Technology / UTOX Environmental Toxicology; H. Youn, Seoul National University / Institute of Health and Environment; S.K. Shin, Seoul National School / Institute of Health and Environment; I.B. Castro, Universidade Federal de São Paulo / Instituto de Ciências Biológicas; M. Fernández Cruz, Instituto de Tecnología y Ciencias del Agua y del Atmosfera (ITACyL); M.J. Suter, Eawag - Swiss federal Institute of Aquatic Science and Technology / UTOX Environmental Toxicology; K.J. Groh, Eawag - Swiss federal Institute of Aquatic Science and Technology / UTOX Environmental Toxicology; F.D. Leusch, Griffith University - School of Environment and Smart Water Research Centre / Smart Water Research Centre; B.J. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; B.I. Escher, Griffith University - School of Environment and Smart Water Research Centre

TH171

Thyroid disruption by triphenyl phosphate, an organophosphate flame retardant, in zebrafish (Danio rerio) embryos/larvae and two cell lines

S. Kim, J. Jung, Seoul National University / School of Public Health; I. Lee, D. Jung, Seoul National School / Institute of Health and Environment; H. Youn, Seoul National University; K. Choi, Seoul National University / School of Public Health.

Triphenyl phosphate (TPP) is one of the most widely used organophosphate flame retardants (OPFRs). Although TPP has frequently been detected in environmental and biota, current knowledge on its toxicological effects is limited. The present study was conducted to understand adverse impacts of TPP on the thyroid hormone system and underlying mechanisms, using early life stage of zebrafish and two cell lines, i.e., rat pituitary (GH3) and thyroid follicular (FRTL-5) cells. In the GH3 cells, TPP up-regulated the transcription of tshβ, treα, and tshβ genes while a positive correlation between the opposite direction of change. In FRTL-5 cells, exposure to TPP led to significant up-regulation of the nis and tpo genes, suggesting that TPP stimulates thyroid hormone synthesis in the thyroid gland. In zebrafish larvae, TPP significantly increased the levels of both T3 and T4 and transcription of the genes involved in thyroid hormone synthesis, at 7 days post-fertilization (dpf). Exposure to TPP also led to significant up-regulation of the genes involved in the transport (tr), metabolism (dio1), and elimination (ugt/l) of thyroid hormones in zebrafish larvae. The down-regulation of the ceh and tshβ genes suggests that the increased T3 levels activate a central regulatory feedback mechanism in vivo. In conclusion, our observations show that TPP could increase the thyroid hormone levels in the early life stages of zebrafish by influencing the central regulation and hormone synthesis pathways.

S. Kim, J. Jung, Seoul National University / School of Public Health; I. Lee, D. Jung, Seoul National School / Institute of Health and Environment; H. Youn, Seoul National University; K. Choi, Seoul National University / School of Public Health; H. Youn, Seoul National University / Institute of Health and Environment; S.K. Shin, Seoul National School / Institute of Health and Environment; I.B. Castro, Universidade Federal de São Paulo / Instituto de Ciências Biológicas; M. Fernández Cruz, Instituto de Tecnología y Ciencias del Agua y del Atmosfera (ITACyL); M.J. Suter, Eawag - Swiss federal Institute of Aquatic Science and Technology / UTOX Environmental Toxicology; K.J. Groh, Eawag - Swiss federal Institute of Aquatic Science and Technology / UTOX Environmental Toxicology; F.D. Leusch, Griffith University - School of Environment and Smart Water Research Centre / Smart Water Research Centre; B.J. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; B.I. Escher, Griffith University - School of Environment and Smart Water Research Centre

TH173

Estrogenicity of environmentally relevant chemical mixtures is not predicted by ethinyl estradiol

H. Yu, C.M. Johnson, Temple University / NSF WET Center; D.J. Caldwell, Johnson & Johnson; R.P. Suri, Temple University / NSF WET Center

Endocrine active compounds (EACs) comprise a wide range of natural and synthetic estrogen hormones, pharmaceuticals, pesticides and industrial chemicals, which may alter the function of endocrine systems and consequently cause adverse effects in wildlife and humans. The presence of EACs in wastewater, surface water, groundwater and even drinking water has become a major public concern worldwide. Exposure to a mixture of EACs has been predicted to result in additive effects, but this has not been studied using environmentally relevant mixtures of EACs. In this study, the estrogenic effects of 11 EACs of high environmental concern were systematically investigated using the yeast estrogen screen (YES) method. The contribution of individual chemicals to the total endocrine activity of environmentally relevant mixtures was evaluated. 17β-estradiol (E2) was used as positive control on each plate in the YES assay. Bisphenol-A (BPA), estrone (E1), estriol (E3), ethinyl estradiol (EE2), and genistein (GEN) showed estrogenic activity when compared with E2. Bis(2-ethylhexyl) phthalate (DEHP), octyl phthalate (OP), nonyl phthalic (NP), Benzyl butyl phthalate (BBP), and Dibutyl phthalate (DBP) showed anti-estrogenic activity. The full mixture of all these chemicals at an environmentally relevant ratio also showed week anti-estrogenic activity. Further, EE2 did not have a prominent contribution to the estrogenic activity of the mixture. We conclude a holistic evaluation of the estrogenic activity is necessary to evaluate the risk of a mixture of EACs.

H. Yu, C.M. Johnson, Temple University / NSF WET Center; D.J. Caldwell, Johnson & Johnson; R.P. Suri, Temple University / NSF WET Center

TH174

Does dissolved organic carbon cause apparent antagonism in reporter gene assays?

S. Wiskow, Griffith University - School of Science and Environment / Future Industry Institute; B.L. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; F.D. Leusch, Griffith University - School of Science and Environment / Future Industry Institute

There is increasing awareness of the importance of assessing antagonism in parallel with agonism for environmental samples, such as wastewater, as a number of EACs have observed both estrogenic and anti-estrogenic effects when using cell-based bioassays. Some studies have attributed this to the presence of dissolved organic carbon (DOC), which is found in surface waters and wastewater and can be co-extracted during sample enrichment prior to bioanalysis. DOC has a high sorption capacity for the estrogen receptor (ER) agonist 17β-estradiol, which may reduce the 17β-estradiol free concentration used in the antagonist testing mode and potentially lead to apparent antagonism. This study applied an experimental and modelling approach to assess the influence of DOC when assessing antagonism using an ER reporter gene assay. Humic acid and fulvic acid were used as positive and negative controls, respectively, in the YES assay. The antagonism of 17β-estradiol was assessed in the presence of increasing DOC concentrations. There was a significant decrease in the antagonism of 17β-estradiol in the presence of DOC. The results were comparable with the modelled reduced 17β-estradiol activity at environmentally relevant DOC concentrations. These results suggest that DOC can cause apparent antagonism in reporter gene assays.

S. Wiskow, Griffith University - School of Science and Environment / Future Industry Institute; B.L. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; F.D. Leusch, Griffith University - School of Science and Environment / Future Industry Institute

TH175

MARINAS AS SOURCE OF FRESH TBT IN SOUTH AMERICA

I.B. Castro, Universidade Federal de São Paulo / Instituto do Mar; G. Fillmann, Universidade Federal do Rio Grande / Institute of Oceanography

Tributyltin (TBT) is a very toxic compound that was used as antifouling paints

I.B. Castro, Universidade Federal de São Paulo / Instituto do Mar; G. Fillmann, Universidade Federal do Rio Grande / Institute of Oceanography

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biocide during more than four decades. Due to the environmental toxicity the International Maritime Organization banned TBT-based antifouling paints in September 2008. Thereafter, TBT environmental concentrations as well as imposex levels (the most widely used TBT biomarker) have been declining in several coastal areas worldwide. However, Brazil and Uruguay are the only signatory countries of the antifouling systems convention and recent studies have shown that TBT concentrations in Brazil are still an important issue along South American coastal areas. Thus, the incidence of organotin and butyltin compounds (BTs) was appraised in three selected areas from South America. Muricid gastropods and surface sediments were sampled in sites under the influence of harbors and marinas from Venezuelan Caribbean Sea (Venezuela); Fortaleza (Brazil) and Lima (Peru). Imposex parameters (% imposex, RPLI and VDSI) and BTs levels were determined and the results were spatially evaluated considering potential TBT sources (harbors and/or marinas). In addition, a time-trend for imposex and BTs levels were evaluated for Fortaleza and Lima. Despite the general pattern of reduction, areas under the influence of marinas always presented high TBT and imposex levels. VDSI and RPLI ranged from 3.8 to 4.6 and 49.4 to 54.6 in marinas of Venezuela, whilst values were much lower for other coastal areas (0.2 to 1.9 and 0.1 to 5.6, respectively). In Fortaleza (Brazil), VDSI and RPLI values were ≤0.92 and ≤0.19 in sites under the influence of harbors and up to 4.2 and 97.0 in areas under influence of leisure boats (marina and yacht club). At Perú, much higher levels of imposex (RPLI = 8.4 – 28.4 / VDSI = 3.3 – 3.5) and TBT in tissues (24-61 to 111-145 ng Sn g⁻¹) were detected in two sampling sites after the establishment of a marina in 2010. Thus, although the international restrictions on TBT use are apparently reducing the inputs from harbors (these the Antwerp Sea; Baía de Guanabara in Brazil and Pó in Peru) we clearly pointed out that marinas are becoming an important source of fresh TBT pollution. Based on that, a similar pattern might be expected for other South American coastal areas.

TH176 Decreased BT levels and changes in Imposex development in Hexaplex trunculus (Gastropoda, Muricidae) from the Northern Adriatic Sea (Italy) F. Cacciatore, ISPRA-Institute for Environmental Protection and Research / Mitigation and Prevention of Impacts; C. Antonini, M. Formalewicz, C. Gion, ISPRA - Institute for Environmental Protection and Research; S. Noventz; D. Berto, R. Boscolo Brusa, ISPRA - Institute for Environmental Protection and Research; M. Marin, University of Padova / Department of Biology Imposex (superimposition of male sexual characteristics on females of gonochoristic gastropods) is an endocrine disruption effect used to indirectly assess the presence of tributyltin (TBT), a once popular biocide in antifouling paints, which has been worldwide banned since 2008. The aim of this study was to monitor imposex concentrations and imposex development in the Northern Adriatic Sea (Italy) in the species Hexaplex trunculus, with DBT and MBT detected (range 2BT: 9 – 16 ng Sn g⁻¹ dw), with DBT almost always higher than TBT levels, except in spring samples where maximum BT concentrations were also detected, probably related to decreases in meat yield linked to spawning season. No relationship was found between shell size and BT body burden, Out of the 392 H. trunculus assessed, 46% were male and 54% were female. Imposex Incidence (%), expressed as the expected frequency of the Relative Penis Size Index (RPSI) of 0.1-8% and Female Penis Length (FPL) of 0-3mm were found. Seasonal and size differences were found only for RPSI and male penis lengths. The development of imposex exhibited a different course relative to that observed in H. trunculus from the same area before TBT ban, when BT concentrations and imposex levels were much higher. Previous studies documented the follow up of the incidence penis, followed by the development of var des. In this study, the onset of imposex occurred with the appearance of the vas deferens halfway between the right ocellar tentacle and vagina. The observed change was supposed to be a consequence of the decreased BT concentrations in the environment.

TH177 Analysis of organotin compounds in antifouling paint particles M. Lagestron, Institute of Applied Environmental Science ITM; J. Strand; E. Ytreberg, Chalmers University of Technology / Department of Shipping and Marine Technology; B. Eklund, Stockholm University / Department of Environmental and Chemical Toxicology Antifouling paints containing organotin compounds (OTC), primarily tributyltin (TBT), have been banned from use on boats shorter than 25m in the EU since 1989 due to their harmful effects, amongst others as an endocrine disruptor, on non-target organisms. However, even though the use of organotin paints has ceased, recreational boats remain sources of OTC to the environment as many have historical coatings of organotin paint on their hulls. Paint particles containing OTC end up on the ground in the boatyards during boat maintenance or in harbour sediments where the OTC are released from the shed particles. As the persistence of biocides from antifouling paints have been shown to be higher in sediments when they are introduced as paint particles, they can act as long-term sources. It is therefore important for risk assessment purposes to be able to accurately quantify the amount of TBT and other OTC in sediments. The extraction efficiency of OTC from paint flakes using an extraction and derivatization method for sediments employed at a lab accredited for butyltin compounds was tested against a modification of the method containing an additional extraction step involving a solvent mixture with a composition similar to that of paint thinner. The comparison showed that the currently recommended modified method but an increased number of organotin species could also be detected. The results from this comparison indicate that quantification of TBT and other OTC in sediments using existing methods may lead to an underestimation, if paint flakes are present in the sample.

TH178 Environmental quality standards for Bisphenol A R.J. Brown, wcia consulting; D. Leverett, WCA-Environmental Ltd; G. Merrington, Environment Agency Bisphenol A (BPA) is used in the manufacture of plastics, has been widely detected in the aquatic environment and there are concerns about its effects on the environment, RPLI valuation health. As such BPA is considered to be a candidate for eventual "priority substance" status under article 16 of the Water Framework Directive (WFD, 2000/60/EC) which will require that EU-wide Environmental Quality Standards (EQSs) are derived. This poster will outline the process used to derive EQS values and uses it to derive PNEC values for BPA based on available acute and chronic aquatic ecotoxicity data. While existing risk assessments (e.g. E.C.2008) and Red as the TBA for the PNEE-Evaluation, there is no toxicological database of available environmental data for BPA. The application of safety factors for deriving EQS values will be discussed taking into consideration available mesocosm data and the potential concerns for low dose effects of BPA. Human health biota and drinking water PNECs of 304 µg/kg and 17.5 µL respectively were also derived using EFSA’s recently revised Tolerable Daily Intake (TDI) of BPA of 5 µg/kg bw/day. The available monitoring data suggests that BPA in the environment would not readily exceed the proposed EQS. Ref. E.C., (2008): Update of the risk assessment of 4,4'-Isopropylidenediphenol (Bisphenol-A), Environment Addendum.

TH179 Developing a hierarchical testing strategy for micropollutants in drinking water regarding potential endocrine disrupting effects J. Kuckelkorn, RWTH Aachen University / Institute for Environmental Research; R. Redelstein, RWTH Aachen University / Albrecht von Haller Institute for Plant Sciences; T. Heide, J. Kunze, S. Malekt, RWTH Aachen University / Institute for Environmental Research / Institute for Environmental and Drinking Water Toxicology; A. Eckhardt, Federal Environment Agency / Drinking Water and Swimming Pool Water Toxicology; H. Hollett, RWTH Aachen University / Institute for Environmental Research; T. Seiler, RWTH Aachen University / Ecosystem Analysis The joint project “Tox-Box – Risk assessment of anthropogenic micropollutants to aquatic life on the basis of the Drinking Water Quality Standard (DWS) for environmental and drinking water assessment” (FKZ 03W08022D) aims for a harmonized, hierarchical test strategy to assess the toxicity of micropollutants that may occur in drinking water by means of the health-related indicator value (HRIV concept). This concept offers five HRIV thresholds (levels ≤ 0.1 µL⁻¹ to ≤ 0.3 µL⁻¹) depending on availability and completeness of toxicological data regarding genotoxicity, neurotoxicity and germ cell damages. As one part of the Tox-Box module “endocrine effects” the Institute for Environmental Research at RWTH Aachen University analyzed and established endocrine activity as an important, additional toxicological mode of action within this concept using a set of bioassays. The in vitro ERα/AR CALUX® assay detected receptor-mediated endocrine activity in the human osteosarcoma cell line U2-OS (≤ 0.9 fraction for mechanistic activation). A second set of bioassays, identified alterations in the steroidogenesis of hormones 17β-estradiol and testosterone with a competitive ELISA and effects on the expression of different steroidogenic genes (CYP11A, 3β3HSD2, CYP17A and CYP19A) using quantitative real-time PCR in the human adrenocortical carcinoma cell line H295R. The in vivo reproduction toxicity assay with the mud snail Potamopyrgus antipodarum provided data on endocrine disruption at an individual as well as population level. Results indicate that the combination product benzo[a]pyrene (BaP), the pharmaceuticals diclofenac and sulfamethoxazole, the flame retardant tris-(1-chloro-2-propyl) phosphate, the herbicides atrazine, 3,4-dichloroaniline (3,4-DCA) and 2,4-dichlorophenol (2,4-DCP), the fungicide tributyltin oxide (TBT0), the radio contrast agent diatrizoic acid and the industrial chemical perfluorooctanoic acid have no affinity to the estrogen and androgen receptor without metabolic activation, even when applied at their water solubility limit. Using an S9-mix BaP, 3,4-DCA, 2,4-DCP and 3,3-dichlorobenzidine (3,3-DCB) showed effects, the latter also without S9-mix. Atrazine, 2,4-DCP and TBT0 caused effects on hormone production in the H295R assay, which can be correlated
with changes in the expression of steroidogenic genes. Batp caused an effect in the reproduction test with P. antipodarum with a LOEC of 40 ng L⁻¹.

TH180 Predicted effect concentrations and distribution of progestins in European aquatic environment
V. Kumar; University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters; A.C. Johnson, CEH Wallingford / Wallingford; R. Grabic, University of South Bohemia in CB / Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenes; H.K. Kroupová, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries and Protection of Waters

Stimuli for the metabolism of steroid hormones in natural waters can be harmful to aquatic organisms. In recent years, concern has been raised about the natural progesterone and its synthetic derivatives (progestins). There are evidences that some progestins can contribute to endocrine disruption in fish and other aquatic organisms. For example, at the lowest tested concentration 0.8 ng/L, levonorgestrel (LNG) reduced egg production of female fathead minnows by about 95%. However, there is almost no detail study on the occurrence and distribution of these chemicals in aquatic environment. In the absence of occurrence data, it is highly desirable to have a range of effect values for progestins in aquatic environment. To address these issues we selected specific candidates based on their reported concentrations in literature data and their likely potential threat to aquatic wildlife. For our study, reported efficient values of LNG and norethisterone were used to predict their concentration in the Czech rivers. As a starting point we used national dilution factor values for European countries. LNG does not appear to pose a widespread threat to European fish reproduction, however in some locations of Romania, Spain and UK known effect concentration (0.8 ng/L) would be reached for brief periods. Furthermore, predicted bioconcentration factor values can be used to derive effect concentration of progestins in water. We found that some progestins would have a tendency to accumulate in fish, when present in the surrounding water at low ng/L levels. Derived effect concentration values suggested that ethynodiol diacetate and desogestrel were most likely to be bioconcentrated in fish up to human plasma therapeutic levels since an environmental concentration of only 2 to 3 ng/L in the surrounding waters was required. Overall, distribution assumed from the eff fluent concentrations could make a significant contribution to improve our knowledge on the presence of these chemicals in European aquatic environment.

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TH181 Quantification of plasticizers by UHPLC-MS/MS and application to a drinking water treatment plant
G. Bolleda, S. Puig, Escola Universitària Salesiana de Sarrià; M. Boleda, Agbar; S. Lacorte, IDAEA-CSIC / Environmental Chemistry

Plasticizers are a group of substances added to plastics to modify their physical properties as flexibility or durability. Phthalates have been used for a long period of time in the industry. Plasticizers are considered as emerging environmental pollutants as a result of their extensive use and their endocrine disrupting properties. These plasticizers are present in plastic pipes, used in industry and also in drinking water treatment plants, and they are capable to migrate from the plastic to water due to temperature and the period of time the water is in contact with this material. The aim of this work was to develop a method for the analysis of 22 plasticizers (dimethyl phthalate, diethyl phthalate, benzyl butyl phthalate, dibutyl phthalate, bis(2-ethylhexyl)adipate, bis(2-ethylhexyl) phthalate, benzoophenone, biphenyl A, 7 butylnonyl isomers, 4-tert-amylphenol, 4-ethylphenol, 4-heptylphenol, 4-octylphenol, 4-nonylphenol, 4-dodecylphenol) by ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) operating in multiple reaction monitoring mode. Solid phase extraction with Oasis HLB cartridges was used to analyse water in the different stages from a drinking water treatment plant: river water, sand filtration, ozonization, carbon active, ultra filtration, reverse osmosis and treated water. Phthalates, BPA, nonylphenol and octylphenol were detected in different stages. Bisphenol A was the only target compound completely eliminated throughout the treatment plant. Other contaminants were present at low μg/L level. Tolerable daily intake (TDI) defined by the European Food Safety Authority, was calculated to estimate the consum of the highest concentration of each compound in the distribution waters analysed within this study, and 2 L water ingestion per day for a 60 kg person. In all cases, values are well below the TD1. Acknowledgement - The authors thank the Ministry of Education, Science and Innovation in Spain (INNPACTO, IPT-2011-0709-06000).

TH182 On the importance of metabolism for identifying potential endocrine disruptors in industrial chemicals - evaluation and development of in silico tools
A. Rzbacka, Chemistry Department; C. Ruden, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; I. Tetko, Helmholtz Zentrum München, German Research Center for Environmental Health; P.L. Andersson, Umeå University / Chemistry Department

Several endocrine disrupting chemicals (EDCs) are known to undergo fast metabolic transformation and some pathways are induced by metabolites rather than by the parent compounds. Experimental metabolite profiles are scarce, but metabolite simulators, which can provide insights and guidance, are available. By predicting possible metabolites, these in silico tools can provide insights for quantitative structure-activity relationship (QSAR) models to assess the effects associated with endocrine activity of untested metabolites. The aim of this study is to improve the identification of EDCs by developing and evaluating in silico tools that predict three types of endocrine alteration: estrogen (E), androgen (A), and transphytrenin (T) binding. In particular, the study focuses on evaluating the use of a metabolism simulator to reduce the number of false negatives predicted by EAT models. A large database was collected to reach a wide model applicability and the developed models were applied on the European inventories of high and low production volume industrial chemicals (H&LPVCs) aiming at identifying potential EDCs. Balanced accuracies of the EAT models ranged from 77-87%, 62-77%, and 65-89% for E-, A-, and T-binding respectively. Of a large set of industrial chemicals to which the models were applied in order to generate predictions, 9% were E- and/or A-binders and 1% were T-binders. The numbers of E- and T-binders increased 2- and 3-fold, respectively, when a metabolism simulator was applied. An evaluation of in vivo data showed that the metabolite simulator was able to correctly identify compounds interfering with thryoxine levels in vivo. Combined use of the EAT models and the metabolism simulator resulted in identification of 50% of industrial compounds present on standard lists of potential endocrine disruptors such as the TEDX[1] and EU priority lists. The data and models used in this study will be publicly available on-line at http://fochem.eu. <br />[1]http://endocrinedisruption.org/endocrine-disruption/text-list-of-potential-endocrine-disruptors

TH183 Exposure Assessment of Phthalate Esters for Chinese Population Based on Exposure Scenario and farm-scenario to Estimate Methods
Y. Cao, J. Liu, X. Hao, Peking University

Phthalate esters are widely used as plasticizers and consequently ubiquitous in environmental media and human bodies. The possible adverse health effects and various exposure pathways made phthalate esters a general concern. On the basis of extensive monitoring data available around China, this study evaluated exposures to: phthalic anhydride (PA), di-(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP) and dibutyl phthalate (DdBP) using exposure scenario method and biomonitoring estimation method at the same time. The median exposure levels from scenario estimation and biomonitoring estimation were 4.36, 2.78 and 1.03 μg/kg bw/day for DEHP, DBP and DiBP, respectively, showing acceptable levels of exposure, compared with the reference dose (RfD) and tolerable daily intake (TDI) values released by the EPA or the EU. Meanwhile, Food is the main exposure source for both DEHP and DBP while indoor air and dust have a significant contribution to the estimated daily intake of DiBP. Regional assessment in several developed areas with large plastic productions obtained higher exposure levels, which should be paid more attention in the context of rapid development of the plastics industry. Keywords: phthalate esters; exposure assessment; exposure scenario method; biomonitoring estimation method

TH184 Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach
V. Berg; Laboratory Medicine; T. Haugdal Nøst, University of Tromsø / Depament of Community Medicine; A. Veyhe, University of Tromsø / Depament of Community Medicine; A. Elverland, University hospital of Northern Norway / Labotary Medicine; T. Haugdahl Nøst, University of Tromsø; J. Odland, UiT The Arctic University of Norway; T.M. Sandanger, UiT The Arctic University of Norway / departement of community medicine

The mechanisms involved in thyroid homeostasis are complex, and perfluoralkyl substances (PFASs) have been indicated to interfere at all levels. Disruption of the maternal thyroid homeostasis during early pregnancy is of particular concern, where subclinical changes in maternal thyroid hormones (THs) may affect early pregnancy and foetal development. The present study investigated TH concentrations between THs, thyroid binding proteins (TH-BPs) and PFAS concentrations in pregnant women from Northern Norway. Women participating in The Northern Norway Mother-Child contaminant Cohort Study (MISA) donated a blood sample at three visits related to their pregnancy and postpartum period (during the second trimester, 3 days and 6 weeks after delivery) in the period 2007-2009. Participants were assigned to quartiles of various PFAS concentrations during the second trimester and mixed effects linear models were used to investigate potential associations between PFASs and repeated measurements of THs, TH-BPs, thyroxin binding capacity and thyroid peroxidase antibodies (anti-TPO). Women within the highest perfluorooctane sulfonate (PFOS) quartile had higher mean concentrations
of thyroid stimulating hormone (TSH) compared to the first quartile at all sampling points. Women within the highest quartiles of perfluorodecanoate (PFDA) had lower mean concentrations of triiodothyronine (T3) and women within the highest quartiles of perfluorooctanoate (PFOA) had lower mean concentrations of free triiodothyronine (FT3). Further, the difference in concentrations and the changes between three time points were the same for the PFAS quartiles. Thyroxin binding capacity (TBC) was assessed as part of THs and TH-BPs, and was selected as a holistic adjustment for individual changes in TH homeostasis during pregnancy. Finally, adjusting for maternal iodine status did not influence the model predictions. In Findings the present study suggest modifications of TH homeostasis by PFASs in a background exposed maternal population. The variation in levels of THs between PFAS quartiles were within normal range ranges and may not be of clinical significance in the pregnant women. However, subtle individual changes in maternal THs may have significant consequences for foetal health.

TH185 Exposure to persistent organic pollutants (POPs) in pregnant women and newborn infants and associated endocrine disruption effects S. Kim, Seoul National University / Department of Environmental Health; J. Park, SOON CHUN HYANG UNIVERSITY / College of Natural Sciences; S. Kim, Seoul National University; H. Moon, Hanyang University / Marine Sciences and Convergent Technology; K. Choi, Seoul National University / School of Public Health Persistent organic pollutants such as polychlorinated biphenyls, polybrominated diphenyl ethers, and organochlorine pesticides are of global concern because of their widespread contamination and adverse health effects. Due to the susceptibility of fetuses to prenatal exposure to POPs, pregnant women are important group which deserves such investigation. In the present study, associations between POPs exposure and thyroid hormones or adipokines levels among the pregnant women and matching children were investigated. We observed that significant disruption of thyroid hormone levels and adipokines levels in pregnant women, and that prenatal exposure to several POPs were associated with decrease of T3/T4 levels and increase of TSH in cord serum and neonatal bloodspot both. In addition, we found the significant influence of POPs exposure to disturbance of adiponectin and leptin production in breast milk. Considering the importance of thyroid hormones and adipokines during gestation and early life stages and the effects of interaction between thyroid hormone and adipokines concentration on obesity or weight loss, health implication of low level POPs exposure among newborns and pregnant women deserve further follow up investigations.

TH186 Internal dosimetry metrics for risk assessment of endocrine disruptors - the case of bisphenol A D.A. Sarigiannis, S. Karakitsios, E. Handakas, A. Gotti, Aristotle University of Thessaloniki / Chemical Engineering A major outcome of INTEGRA project (CEFIC LIF1) is a computational platform that integrates multimedia environmental, (external) exposure and toxicokinetic modeling within a dynamic framework in time. Use of toxicokinetic modelling transforms exposure/risk assessment of environmental chemicals since it allows risk characterization to be based on internal dosimetry metrics. In this way high throughput system data such as the ones generated by Tox21 in vitro testing can be used, towards the nowadays need of “exposure based risk assessment”. This opens the way towards a higher level of assessment that incorporates refined exposure (tissue dosimetry) and toxicity testing (Biological Pathway Altering Dose, BPAD). The applicability of INTEGRA was tested on bisphenol-A. Several exposure scenarios were investigated, (a) incorporating data from external exposure assessment based on food residues and food consumption patterns, (b) from exposure reconstruction of real life EU-wide human biomonitoring data using exposure reconstruction algorithms. Reconstructed exposure was then run in forward mode in the PBPK model, so as to estimate the Biologically Effective Dose (BED) in the target tissue. In order to associate the risk of the several exposure scenarios based on BED derived by the PBPK model, two different exposure metrics were used: - The Tolerable Daily Intake (TDI) of 50 μg/kg bw/d proposed by the European Food Safety Authority, was translated into internal exposure, forming corresponding concentration of free plasma BPA. In vitro, the ToxCast assays provided six ER agonist or binding AC50 values for BPA, ranging from 0.6 to 1.7 μM. To calculate a conservative Biological Pathway Altering Dose (BPAD), the lowest ToxCast AC50 was selected (0.64 μM for Attigane Factorial cis ERE assay). This estimated concentration (145 μg/l) is 3 orders of magnitude higher than the equivalent derived from the EFSA TDI (0.16 μg/l) of the hypothesis that taking into account age dependent bioavailability differences of BPA, specific exposure scenarios (i.e. bottle fed neonates and premature infants hosted in intensive care units) result in BED close to the EFSA TDI legislative threshold. Use of the ToxCast AC50 BPAD as an internal exposure risk characterization metric, resulted in increased margins of safety compared to conventional exposure/risk characterization.

TH187 Classification of the Ecological Quality Status of the Portuguese coast concerning TBT pollution, based on the levels of imposex in two gastropod species. F. Laranjeiro, Biology Department CESAM; P. Sanchez Marin, University of Aveiro & CESAM / INRSETE; J.B. de Carvalho Benta Santos Oliveira, University of Aveiro & CESAM / Biology department; S. Galante-Oliveira, C. Barroso, University of Aveiro / Biology Department CESAM The Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSDF) were developed in the European Union in order to protect aquatic ecosystems. One of the most difficult concepts in these directives was the pollution by contaminants like tributyltin (TBT), a biocide largely used in antifouling paints in the past and considered the causative agent of imposex in gastropods, as well as other known deleterious effects. In order to integrate TBT pollution assessment within these legislative frameworks, Ecological Quality Ratios (EQR) were developed for imposex assessment in two different gastropod species of the dogwhel Nucella lapillus and the netted wink S. auricularia reticulata. Those EQRs were then applied to an imposex monitoring campaign performed in 2014 along the Portuguese coast, in order to classify the Ecological Quality Status. According to our proposal, the levels of imposex observed in Nucella lapillus indicate that all assessed sites present a Good Ecological Quality status. The same conclusion is obtained when using the proposed EQR by the WFD-United Kingdom Technical Advisory Group (WFD-UKTAG) in this species. On the other hand, imposex levels exhibited by the netted whelk showed that some stations present a Moderate to Poor/Bad Ecological Quality Status, namely the ones located in fishing ports and dockyards. As TBT is listed as a priority substance in the WFD Annex X, the monitoring of this compound should be performed regularly. Our combined approach involving these two species is an effective and low cost tool to assess the environmental quality of the Portuguese coast.

TH188 The planar-YES bioassay: a new approach to measure the effects of oxidation procedures on surface water samples A. Schönborn, A.A. Grimmer, ZHAW The planar-YES ([2],[3], a variant of the yeast estrogen screen (YES) [1]) was used to assess the efficacy of three different oxidation procedures for the removal of microcontaminants from potential drinking waters. The procedures were: a) ozonation, b) chlorination, and c) advanced oxidation (AOP) using hydrogen peroxide and ozone. The raw water samples were divided into two lines, one of them left native and the other being spiked with 9 selected microcontaminants found in Swiss surface waters. The two samples were then subjected to each of the 3 oxidation procedures, with 8 samples being taken before and after oxidation. The results in 12 different measurements per sample. All samples were then extracted using solid phase extraction. We showed that all 3 oxidation processes are able to remove a significant part of estrogenic activity from the water, but traces still remain active. We also showed that chlorination can lead to a small but significant increase of estrogenic activity in treated surface water after the procedure. The reasons for this are currently still unknown. Our work also lead to a significant improvment of the planar-YES method, namely in the application technique of the yeast culture to the HPTLC plates. The planar-YES is an interesting tool for the assessment of estrogenic activity in aqueous samples of various kinds (e.g., surface waters, recycled water, water from fish culture, water stored in plastic packages etc) and should receive more attention in the scientific community. It offers a way to combine and effect-directed analysis is available from the use of the liquid chromatography with other strategies. Masses as low as 0.5 pg 17-beta-estradiol equivalents per spot can be detected. The method has the potential to avoid solid phase extraction by allowing the application of native water samples directly on plate. We are convinced that the limit of detection has still not yet been reached. The next steps of our research will focus on the direct detection of estrogenic substances in TCL-bands by using TOF-MS. References [1] E.J. Routledge, J.P. Sumpter, Environ. Toxicol. Chem. 15 (1996), 241–248. [2] A. Schönborn, A. A. Grimmer, Journal of Planar Chromatography 26 (2013) 5, 402–408 [3] S. Buchinger, D. Spira, Journal of Planar Chromatography 26 (2013) 5, 395-401

Carbonaceous materials for contaminants remediation (P)

TH189 Interactions between unconventional biocarbons and organic contaminants M. Keh, University of Vienna / Department of Environmental Geosciences; G. Spurny, H. Sun, University of Vienna; T. Hofmann, University of Vienna / Department of Environmental Geosciences Biocarbons can exhibit great sorption potential towards heavy metals and organic contaminants, and have therefore been proposed to sequester contaminants for remediation purposes. Most biocarbons studied so far were derived from plant residues, and little knowledge is available for biocarbons derived from waste materials and that are characterized by high mineral contents (e.g., sewage sludge, manure). Using wastes as feedstock to produce biocarbons would support the development of attractive combined strategies for waste management and remediation. High mineral contents are expected to strongly affect sorption behaviour, especially that of compounds interacting through specific mechanisms of sorption (e.g., polar,
ionizable compounds). In this study, single point sorption coefficients for a series of model sorbates were measured onto a series of biochars produced at 200°C, 350°C and 500°C. Feedstock materials consisted of wood shavings (yielding biochars with < 1% ash), pig manure (biochars with 30-60% ash) and sewage sludge (biochars with 50-70% ash). A commercial biochar derived from grain husks was also included (40 % ash) and sorption to a commercial activated carbon was used as a benchmark. For a selected subset of materials, detailed experiments were also carried out to investigate the effects of particle size fractions and acid demineralization. Harsh extraction with organic solvent indicated acceptable contents of inherent polycyclic aromatic hydrocarbons (within the guidelines values proposed by the International Biochar Initiative). Sorption affinity strongly depended on the biochar – sorbent combination and covered an extremely wide range of values (0.1 < Log Kd < 6.5 L/kg). All materials were extensively characterized (e.g., elemental composition, surface area and porosity) and multivariate analyses were applied to identify the factors driving the great discrepancies in sorption observed. Overall, we demonstrate that (i) biochars derived from animal wastes have potential for remediation applications, and that (ii) describing the interactions between unconventional biochars (e.g., animal waste) and sorbates (e.g., ionizable compounds) requires the consideration of parameters that are typically not accounted for (e.g., pH).

TH190 Sorption of halogenated phenols and pharmaceuticals to biochar: Affecting factors and mechanisms
S. Oh, University of Ulsan / Department of Civil and Environmental Engineering; Y. Seo, University of Ulsan
The feasibility of using biochar as a sorbent to remove nine halogenated phenols (2,4-dichlorophenol, 2,4-dibromophenol, 2,4-difluorophenol, 2-chlorophenol, 4-chlorophenol, 2-bromophenol, 4-bromophenol, 2-fluorophenol, and 4-fluorophenol) and two pharmaceuticals (triclosan and ibuprofen) from water was examined through batch experiments. Types of biochar, synthesized using various biomasses including fallen leaves, rice straw, corn stalk, used coffee grounds, and biosolids, were evaluated. Compared to granular activated carbon (GAC), most of the biochar samples did not effectively remove halogenated phenols or pharmaceuticals from water. The increase in pH and deprotonation of phenols in biochar systems may be responsible for its ineffectiveness at this task. When pH was maintained at 4 or 7, the sorption capacity of biochar was markedly increased. Considering maximum sorption capacity and properties of sorbents and sorbates, it appears that the sorption capacity of biochar for halogenated phenols is related to the surface area and carbon content of the biochar and the hydrophobicity of halogenated phenols. In the cases of triclosan and ibuprofen, the sorptive capacity of biochars (GAC) was significantly affected by pH, according to the point of zero charge (PZC) of sorbents and deprotonation of the pharmaceuticals. Pyrolysis temperature did not affect the sorption capacity of halogenated phenols or pharmaceuticals. Based on the experimental observations, some biochars are good candidates for removal of halogenated phenols, triclosan, and ibuprofen from water and soil.

T. Gecht, University of Tuebingen / Institute for Experimental and Clinical Pharmacology and Toxicology; M. Obst, University of Tuebingen / Center for Applied Geosciences
Polycyclic aromatic hydrocarbons (PAHs) are among the most important pollutants since high concentrations of this compound group occur at many contaminated sites (e.g. former gaswork sites, harbor sediments, etc.). Carbonaceous materials such as soot, charcoal, unburned coal and kerogen exhibit very strong (sorption) affinities for PAHs. Consequently, activated carbon has been applied at contaminated sites such as harbor sediments to sequester pollutants. However, the efficiency of such amendment strategies may be affected by sorption of other compounds such as dissolved natural organic matter (competitive sorption). Today it still remains an open question how ageing of carbonaceous materials, subsequent to mixing into soil or sediment, changes the sorption properties of biochars and activated carbon. For our study we used scanning transmission X-ray-microscopy (STXM) to visualize the distribution of phenanthrene as a model compound for PAHs among different treatments of activated carbon. Prior to the phenanthrene sorption batch experiments, the sorbent was treated with oil or humic acid, respectively. Phenanthrene as well as the organic co-sorbents (oil, humic acid) was mapped on activated carbon by means of their XANES (near edge X-ray absorption fine structure) fingerprints. The results revealed reduced sorption of phenanthrene in case of the humic acid treatment and the visualization and quantification with STXM showed that humic acid accumulates mainly at the outer surface of the activated carbon particles, whereas phenanthrene migrates into the intraparticle pore space. This suggests reduced diffusion of phenanthrene into the particles due to the surface coverage by humic acid. In contrast, similar distributions of phenanthrene and oil were observed in case of the oil-treated activated carbon, indicating that the oil may either compete with phenanthrene for the same sorption sites, or provide an additional sorption domain within the particles for hydrophobic organic contaminants (i.e., partitioning of phenanthrene into the oil within the intraparticle pore space).

TH192 Photocatalytic degradation of methylene blue dye in aqueous solutions by pyrolytic tyre char/TiO2 composites
V. Makrianni, Unit of Patras / Department of Environmental and Natural resources Management; A. Giannakas, C. Daikopoulos, Y. Deligiannakis; University of Patras; L. Konstantinou, Department of Environmental and Natural resources Management
In this work the preparation of TiO2/tyre rubber pyrolytic char (PC) composite photocatalysts via a simple sol-gel method was studied. The photocatalytic performance of the PC/TiO2 catalysts has been assessed for the photodegradation of methylene blue aqueous solutions in order to evaluate the possible synergistic effect of PC and optimize the (wt. %) char/TiO2 ratio. Catalysts with different weight ratio of Char (C) to Ti (T) were prepared as follows: C0.2/2, C0.5/2, C0.7/2, C1.0/2 and Ti0-blank. Anatase TiO2 crystal phase was obtained for all the composite catalysts and traces of brookite crystal phase were formed in TiO2char (@ 4%) and CT 1.02 (@10%) as determined by XRD analysis. The average crystal size of anatase TiO2 (d002) ranged from 13 to 20 nm. The surface areas (S BET) for composites catalysts are proportional to the amount of char in the catalysts and ranged from 43.96 to 127.2 m2g-1 for the PC/TiO2 catalysts CT 0.22 - CT 1.02, respectively. Electron Paramagnetic Resonance (EPR) spectra were recorded at 77K with a Bruker ER200D spectrometer, equipped with an Agilent 3510A frequency counter operating at the X band. The irradiation of methylene blue dye on the catalysts was carried out using a Xenon XLS + apparatus (Atlas, Germany) simulating natural sunlight irradiation. The composite catalysts PC/TiO2 exhibited an improved photocatalytic degradation of MB in comparison with pure-TiO2. The photocatalytic degradation rates of MB by the prepared composite photocatalysts, followed apparent first-order kinetics. The CT 0.2/2 catalyst showed the highest photocatalytic efficiency and nearly complete degradation was achieved within 240 min of irradiation. The EPR analysis showed that incorporation of TiO2 into PC results in faster photokinetics of ‘OH generation in solution due to the enhanced ‘h generation in the TiO2 crystal while the pyrolytic carbon matrix acts as a sink for the photoinduced electrons which migrate from the TiO2 conductive band. This results in an increased h+ lifetime due to diminished hole-electron recombination. Acknowledgement - This work is financially supported by the ‘SYNERGASIA’ Program 11SYN_5_682 (O.P. Competitiveness & Entrepreneurship (EPAN II), ROP Macedonia- Thrace, ROP Crete'n and Aegean Islands, ROP Thessaly- Mainland Greece- Epirus, ROP Attica.

TH193 Effect of grain size and heavy metals on As immobilization by pyrolytic graphite
M. SIMON TORRES, I. GARCIA FERNANDEZ, University of Almería / Agronomy Department; V. Gonzalez, Laboratoire interdisciplinaire des environnements continentaux LIEC CNRS UMR; A. Romero Freire, F.M. Peinado, University of Granada / edafologia y quimica agricola
The effect of grain size and heavy metals on As immobilization by pyrolytic graphite was determined. The sorption capacity of pyrolytic graphite (1% As) was evaluated on a series of soils. The sorption capacity of pyrolytic graphite was markedly increased when the pH was increased. The sorption capacity of pyrolytic graphite was related to the surface area and carbon content of the graphite and the hydrophobicity of pyrolytic graphite. In the cases of heavy metals (Cd, Cu, Ni, Pb, Zn) the sorption capacity of pyrolytic graphite was significantly affected by pH, according to the point of zero charge (PZC) of sorbents and deprotonation of the heavy metals. Pyrolysis temperature did not affect the sorption capacity of pyrolytic graphite. Based on the experimental observations, some biochars are good candidates for removal of halogenated phenols, triclosan, and ibuprofen from water and soil.

TH194 Biochar as an alternative to activated carbon for polluted soil remediation
B. Pipirova, VSB - Technical university of Ostrava; T. Natal da Luz, University of Coimbra / Department of Life Sciences; J.M. Sousa, Faculty of Engineering of Porto / Chemical Engineering; X. Domene, CREAF-Universitat Autonoma Barcelona / Unit of Ecology
Biochar is a carbonaceous solid product resulting from biomass pyrolysis, which is due to its large surface area, cation exchange capacity, hydrophobicity and limiting capacity considered as promising potential tool for reducing the bioavailibility of...
both organic and inorganic contaminants. This is why a role of biochar in polluted soil remediation has been proposed, either due to a decreased bioavailability of pollutants that reduce the possible accumulation and toxicity to organisms, but also due the potential enhancement of organic pollutant’s biodegradation by stimulation of microorganism’s activity. Since biochar production is expected to increase in the next future, the aim of this paper was to assess the usefulness of biochar for remediation purposes as a suitable regenerable activated carbon. Springtails (Folsomia candida) were used as bioindicators to study the reproduction improvements as an indication of remediation efficiency. Three polluted soils from Czech Republic, Spain and Portugal with contrasting pollutant composition (mostly Cr, Zn, Pb, Cu and BTEX) were amended with two biochars (produced by slow pyrolysis and gasification, both obtained from the same pine wood) and submitted to allotoredial assay (at application rates of 5% (control), 5 and 10% (w/w)). The models derived for F. candida reproduction suggest that, in the only soil where remediation was significant (Czech Rep.), both biochar and activated charcoal were able to reduce heavy metal bioavailability, at least partly due to their limiting capacity in this acid soil. In two other soils, no evidence of remediation capacity for biochar and also activated charcoal was observed. Furthermore, some of the biochars were shown to cause toxic effects themselves when applied at high rates. The contrasting results observed in different polluted soils point out the need for further investigation, but also the need for an acute selection of the most suitable biochars for each remediation scenario.

**TH197**

Bridging the gaps between analytical and effects-based characterization of biochars as remediation agents

X. Domenec, CREATIF-Universitat Autonoma Barcelona / Unit of Ecology; A. Bastos, University of Averio / Department of Biology ans CESAM

Biochar is the carbonaceous product of biomass pyrolysis, intended for application to soils as a cross-cutting strategy to improve soil functions and sequester carbon. A major limitation role in remediation of contaminated or polluted soils has been indicated. Present scientific and societal interest in combining soil/environmental benefits with pollutant mitigation, biochar as a potential emerging soil contaminant or a source of contaminants has been recently addressed. The feedstock and pyrolysis technology define the physical-chemical characteristics of the resulting biochar, leading to a wide variety of materials. The same factors determine the pollutant burden in biochar, as well as the bioavailability of such compounds over time. Large-scale application of poorly characterized biochars may lead to detrimental impacts overcoming the expected environmental benefits, something that might be prevented by the eco(toxico)logical risk assessment of biochars at relevant application rates. Current biochar standardization efforts in the EU and the US are vital for quality assessment and control. While much progress has already been achieved in defining biochar physicochemical quality criteria, effect-based approaches bridging analytical characterization and observed effects on exposed biochar are generally omitted. Bioassays are effect-based tools able to assess biochar’s bioavailable fractions (rather than total contaminant load) and possible synergistic or antagonistic interactions between concurrent contaminants. Further, they enable evaluating positive effects of biochar by itself or their efficiency as remediation agent once applied to soil. A selected set of state-of-the-art methodologies developed for ecotoxicological assessment of chemicals is presented and their possible contribution discussed in the context of predictive (i.e. characterization) as well as of retrospective (i.e. ecotoxological effects) risk assessment of biochar. Using standard statistical and biological tools, one can effectively understand, predict, manage or mitigate the potential environmental risks associated to large scale biochar applications.

**TH198**

Adsorption of endocrine disruptors using industrial wastes based materials

J. Souza, P. Freitas, Universidade Federal de Minas Gerais / Chemistry; M. Rosmaninho, Universidade Federal de Minas Gerais / Quimica; L.D. Almeida, T.R. Santos, Universidade Federal de Minas Gerais / Chemistry

The endocrine disruptors represent a new type of waste that has aroused interest due to their effects being harmful to the environment in even low concentrations that they are usually found. Among the different techniques proposed to remove such residues, bioaccumulation in biota and consistently biomagnifies through the food chain. Mercury can be emitted in a natural way like volcanoes and anthropogenic way. Due to anthropogenic mercury emissions, the concentrations of mercury are three times higher than in pre-industrial days. Among all mercury anthropogenic sources, power plants burning fossil fuels are estimated to account for about 25% of the global anthropogenic mercury emissions. The mercury can enter the environment by direct air injection (ACI) are used for mercury capture, but large amount of toxic wastes are obtained in this abatement process. The objectives of this work are the development of a waste free process for mercury capture. This process is based on a novel gold nanoparticles/carbon regenerable sorbent and consists of gold nanoparticles supported onto structured carbon monoliths. Mercury capture capacity and efficiency have been evaluated in the gas phase using different mercury species formed by inductively coupled plasma (ICP), scanning electron microscope (SEM), filament emission scanning electron microscope (SEM-Fe), X-ray photoelectron spectroscopy (XPS). Sorbents were tested in a bench scale experimental installation working at high space velocities 25000 h⁻¹ and at Hg inlet concentration of 100 μg Hg/m³. Mechanism of mercury retention is the amalgamating and deamalgamating property of mercury for gold. Two different retention temperatures (50 °C and 120°C) and two different atmospheres (N₂ and CO₂) have been evaluated. The regenerability has been tested performing cycles of capture-regeneration at 250 °C. High mercury capture efficiencies were obtained for the sorbents tested, with low gold content, indicating that this novel regenerable sorbent is a promising material for mercury capture in a
waste free process. Moreover, the mercury capture capacity doesn’t depend on temperature allowing the use of these sorbents at different positions on the power plants.

TH199
The influence of pH on adsorption of arsenic from aqueous solution by amino-functionalized multi-walled carbon nanotubes
J. Trklovic, S. Maletic, M. Kraguje, M. Watson, University of Novi Sad Faculty of Sciences; M. Prica, University of Novi Sad Faculty of Technical Sciences / Department of Graphical Engineering and Design; S. Roncevic, University of Novi Sad Faculty of Sciences; B. Dalmacija, University of Novi Sad, Faculty of Sciences / Department of Chemistry, Biochemistry and Environmental Protection
This study demonstrated that it might have beneficial effects to immobilization of As(III) from water on amino-functionalized multi-walled carbon nanotubes (AF-MWCNT). Since both As(III) chemistry and the surface chemistry of AF-MWCNT depend upon the pH, adsorption was investigated at three different pH values (3, 6 and 11). Adsorption experiments were performed using a conventional batch technique at room temperature. The background solution was 0.01 M CaCl2 in deionized water. The amount of AF-MWCNT in each experiment corresponded to a sample/solution ratio that resulted in 20-80% uptake of As(III). The initial concentration of As(III) was in the range 0.6 - 3 mg L⁻¹. The pH was adjusted by HNO3 or NaOH solution. The samples were agitated on a horizontal shaker for 30 h. The time to reach adsorption equilibrium was obtained from a kinetic study performed over a period of 72 h. After reaching equilibrium, the adsorbent was separated from the clear supernatant taken, filtered using a 0.22 µm filter, acidified with HNO3, and analyzed by atomic absorption spectrophotometry (Perkin Elmer AAnalyst 700, USA). Kinetics data followed pseudo-second order model (R² > 0.979), indicating that the adsorption mechanism of As(III) on AF-MWCNT can mainly be attributed to chemical interactions between the surface functional groups and As(III) species. Using the Weber-Morris model, it was determined that intra-particle diffusion contributes to the adsorption of As. However, in all cases, the nonzero intercepts of the Weber-Morris plots were a clear indication that although intra-particle diffusion is slow, it is not the slowest of the rate processes that determine the overall order. The interaction between As(III) species and the surface of the AF-MWCNT remains the most significant rate process. Adsorption capacity, calculated using the Langmuir adsorption model, decreased as pH increased from 3 to 11 from about 40 mg g⁻¹ to about 11 mg g⁻¹, indicating that adsorption is highly influenced by the pH value of the solution. Acknowledgement: This work has been produced with the financial assistance of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project III43005). The project is co-financed by kind support of Provincial Secretariat for Science and Technological Development of the Autonomous Province of Vojvodina (Grant no. 114-451-1158).

TH200
Use of nanoparticles in environmental remediation - the effects of carbon nanoparticles in pharmaceuticals toxicity
R. Faria, R.C. Oliveira, Universidade de Brasília / Department of Genetics and Morphology; D.S. Moura, Universidade de Brasilia / Genética e Morfologia; J.T. Souza, University of Brasilia / Departamento de Genética e Morfologia Instituto de Biologi; N.O. Farias, R.C. Oliveira, Universidade de Brasilia / Gtx / Departamento de Genética e Morfologia Instituto de Biologi; C.K. Grisolia, University of Brasilia / Departamento Genetics and Morphology
Activated and treated carbon nanoparticles are under constant pressure due to human activities that among other impacts can cause pollution and degradation of water and soil resources. The remediation of the toxic effects of pollutants is essential in order to maintain the quality of the environment and hence the welfare and human health. This project brings an innovative approach that integrates the two important fields of science - nanotechnology & environment - in order to obtain the terrestrial and aquatic ecosystems impacted by emerging pollutants, drugs, especially psychiatric medications and antimicrobial. Nanotechnology is a current, high interest-promotes technological knowledge associated with particles having at least one dimension on the gauge scale (< 100 nm) science. Nanotechnology may also contribute to the sustainable development of the human society through new tools for environmental monitoring and even new methods of treatment of complex mixtures. Carbon nanoparticles and metal-oxide nanoparticles are the objects of study in this project, because these have been proposed for decontamination of environmental samples contaminated with organic and inorganic compounds. Detection of several drugs of human and veterinary use in natural ecosystems has called attention of government authorities and the scientific community. The objective of this project was to achieve a bold set of tools for diagnosis and analysis of the environmental effects of water contamination by antimicrobial and psychiatric drugs using zebrafish as biosensor. Thus, scientific bases that can generate protocols and processes for the remediation of soils and waters contaminated by drugs will be produced.

TH201
Activated Carbon in Sediment Remediation - Adverse Effects vs. Remediation Potential
S. Abel, University of Eastern Finland / Department of Biology; I. Nybom, Department of Environmental Biology; S. E. Hale, Norwegian Geotechnical Institute / Environmental Engineering; G. Cornelissee, NGE; F. Akkanen, University of Eastern Finland / Department of Biology
The in-situ treatment of contaminated aquatic sediments with activated carbon (AC) has been shown to be highly efficient in reducing mobility and bioavailability of persistent organic pollutants. However, it has also been found that the sorbent material itself might have negative effects on benthic organisms. This study deals with the question if there are suitable ways to reduce the adverse effects of the sorbent material on the benthic community while still maintaining its high contaminant binding potential. Three different variants of AC (different manufacturers and raw materials), two different particle size ranges, as well as different application methods (mixing with sediment and thin layer capping) were assessed. Additionally it was tested, if the addition of clay to the sorbent material has a significant impact on its performance. Lumbricus variegatus was chosen as a model organism. Both natural, field-contaminated (PCB’s) sediment and clean artificial sediments were used as substrate. The organism’s vital functions were monitored to assess the toxicity of the sorbent materials. The sorbent’s effect on PCB tissue concentration was observed to evaluate the remediation potential. The results showed that AC mixtures into the sediment could lower PCB bioaccumulation in the tissue of L. variegatus significantly already at doses of 0.1%, while AC doses of 1.0% led to a reduction of more than 90%. Correspondingly a significant impact on the test organism’s vital function was observed in the presence of AC particles. Feeding rates dropped drastically in the 0.1% dose and with 1.0% of AC in the sediment a near-cess of any feeding activity was observed. This was accompanied by reduced growth (0.1%AC) and even a loss of biomass (1.0%AC) over an exposure time of 28 days. The three tested AC variants did not differ significantly in their performance. Similar adverse effects were observed when AC was applied as a thin layer cap. The presence of the sorbent generally led to a significant loss in biomass over 28 days. Unlike when mixed into the sediment, the AC dose (cap thickness) had no significant impact on adverse effects. The severity of the reduction in growth was only affected by the AC particle size.

TH202
Effects of compost as an organic carbon amendment to reduce DDT and dieldrin bioavailability to native earthworms
M. Anderson, Civil and Environmental Engineering; A. Torres, University of Maryland / Civil and Environmental Eng; L. McConnell, USDA Agricultural Research Service / Environmental Management and Biporoduct Utilization Laboratory; N.A. Andrade, University of Maryland / Civil and Environmental Engineering; R. Chaney, C.J. Hapeman, USDA-ARS
Historic soil contamination as a result of agrichemical application is a common occurrence throughout the world. Aromatic compounds such as dieldrin and dichlorodiphenylchloroethane (DDT) are hydrophobic organic contaminants and persist in the environment long after application, particularly in soil. The present study investigated bioavailability reduction as a remediation strategy for an aged contaminated orchard site. Following a laboratory scale pot study, two types of local compost were chosen as organic carbon amendments for a single study in an orchard with measurable DDT and dieldrin applications. In the initial pot study, five organic amendments were evaluated, and the two chosen poultry manure comports showed the greatest decrease in earthworm bioavailability over a 45 day incubation period. In the current research, portions, or plots, in the contaminated site had silt fence barriers installed to restrict earthworm movement but allow normal hydrological activity. The silt fence was used to create a total of seven plots divided into 4 sub-plots each. Each sub-plot was randomly assigned one of four treatments: each of the two composts, which were tillied into the soil, on non- amended tilled area, and an undisturbed section. Contaminant concentrations in the soil and native earthworms were determined at three time points over one year to evaluate any changes in bioavailability over time.

TH203
Environmental remediation using carbon nanostructured compounds: the toxic effects of caffeine
N.O. de Farias, Departamento de Genética e Morfologia; R. Oliveira, Universidade de Brasilia / Department of Genetics and Morphology; C.K. Grisolia, University of Brasilia / Department Genetics and Morphology
The occurrence of caffeine (CaF) in natural waters has drawn attention of regulators and researchers worldwide. Caffeine is present in a wide variety of aquatic environments, including wastewater treatment plant effluents, surface waters, groundwater and even remote mountain lakes. For these reasons, there is an immediate concern to understand how this compound may cause affect natural ecosystems acting as a toxic agent. Nanotechnology emerged as an alternative to remediate the toxic effects of organic and inorganic contaminants. In this context, the nanostructured carbons compounds (NCC) are highlighted as an alternative for remediation due to its ability adsorb pollutants. The aim of this study was to
evaluate the application of NCC in the remediation of cacitotoxic effects. To do so, in a first step, the acute toxic effects of CaF and CNC were evaluated separately, using zebrafish embryos as bioindicators. The OECD Protocol no. 236 "Fish Embryos Toxicity (FET) test" was used to assess the lethal and embryotoxic effects of seven treatments of CaF, 0, 30, 45, 53, 68.2, 102.6, 154.5, 232.5 mg/L. The test was extended to 7 days of duration. For NCNs, a test was performed with two different particles, CNC 824 and CNC alfa, in a single exposure dose of 100 mg/L. In the first treatment the embryos were exposed to the carboxylic acids after filtration with both NCNs. All tests were incubated in a climatic chamber at 26 ± 2 °C, 12 h of light. The results of this study demonstrate that extend fish embryo toxicity test is a powerful tool for assessing the toxicity of CaF. Despite the low toxicity of CaF (74-LC50 = 106.0 ± 35.5 mg/L) several abnormalities in development were observed in embryos exposed to 10 mg/L. Thus, the effect of the embryonic test indicates that in chronic exposure with lower CaF could affect embryo-larval stages of fish in natural ecosystems. The NCNs did not cause significant lethal effects on zebrafish embryos. Also, do not interfere with embryonic development, causing only a slight inhibition in hatching after 48 hours of exposure. In summary, the results obtained reinforce the potential of these new NCNs to develop innovative nanotechnology products for water remediation. Future studies might focus on predictable models of toxicity that can be applied in the development of protocols and processes of remediation of contaminated waters.

**TH204**

**Soil nematode communities as indicators of medium-term ecological impacts of biochar addition to agricultural land**

X. Domene, CREA-Universitat Autonoma Barcelona / Unit of Ecology; S. Mattana, CREA; S. Sánchez-Moreno, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) / Unidad de Productos Fitosanitarios

Agricultural soil mesocosms were amended in 2011 with a pine chip gasification biochar (0, 12 and 30 t ha), fertilized with pig slurry, and cropped to barley. After two years, soil nematode communities as indicator of ecosystem impacts were assessed. Soil samples were taken in May and October 2013 and nematodes extracted in Baermann funnel. Nematodes were counted and identified to genus or family under microscope, in order to assess taxa richness and to calculate the Maturity Index. Furthermore, each individual was assigned to a feeding guild (bacterial feeders, fungal feeders, plant parasites/herbivores, omnivores, and predators) for the calculation of soil food web indices (Structure Index, Channel Index, Basal Index, and Enrichment Index). The bacteria-to-fungi ratio was also determined in each sample by qPCR. Of the 31 taxa identified, only the basal bacterivore Cervidellus was significantly affected by biochar addition, with higher abundance in the 30 t ha rate in both samplings. The bacterivore Acroboloeides and the fungivore Trichoscolex presented a significant decrease in sampling x biochar rate. Globally, the bacterivore relative abundance increased with biochar addition, while the abundance of herbivores and predators decreased with the biochar rate. Ecological indices were not affected by biochar addition and only differed between samplings (taxa richness, the Enrichment Index, and the Structure Index). While in May the soil food web condition was similar in all the biochar treatments, in October a more degraded condition was observed at the higher biochar rate. In summary, some negative effects of the addition of this pine gasification biochar were observed at the highest application rate, clearly over the usual agricultural amendment rates but in the range of rates used for soil restoration or remediation purposes. The results from this study point out the need to include ecotoxicological tests in biochar quality assessment guidelines, as well as field studies, to ensure the environmental benefits of this technology and the accurate selection of biochars to be used in soil.

**TH205**

An integrated approach to assess the aquatic effects of biogenic and petrogenic activated carbon used for remediation purposes

A. Mucken, NIVA / Ecotoxicology and Risk Assessment; M. Schaaning, Norwegian Institute for Water Research; A. Lilligrap, NIVA / Ecotoxicology and Risk Assessment

Activated carbon (AC) has long been associated with the capacity to effectively adsorb contaminants from water and air for removing dioxins or dioxin like compounds from contaminated sites. However, the use of AC for remediation purposes has drawn some concern due to possible impacts on benthic dwelling organisms. Within the inner Oslofjord, the use of AC has been well documented for activating carbon (AC) for wastewater treatment due to their unique physical-chemical properties. The question of their fate and impact on the environment has become a major concern due to an expected rise in the production of nanocarbons. Through this study, the potential environmental behavior and impact of the fate of pollutants remains controversial. Nevertheless, research in the field of environmental fate of NCC has been increasing dramatically. However, the data are comparable only to some extent as for all CNM (i) different probe sorbates were used in the study and (ii) various humic substances (HS) were chosen (i.e., kinds or concentrations). Regarding sorption of hydrophobic organic compounds (HOC) and HS by CNM there are two major mechanisms that have been considered: (a) sorption of either HOC or HS as single solutes by CNM and/or (b) competitive sorption of HOC influenced by HS. For the latter, two main mechanisms have been proposed how HS may affect HOC sorption. Either, HS directly competes with HOC for sorption sites on CNM surface, which seems especially relevant as no competitive sorption between HS and either HOC or HS. As a result, HS is reduced in concentration and may otherwise be available for HOC sorption [1,2]. Furthermore, sorption of HOC to HS bound to CNM has to be taken into account despite the fact that this is typically much weaker than on CNM itself. To systematically study this process, three alkane pairs were selected as molecular probe sorbates. The influence of HS on HOC sorption, affinity linearity, and the dominant sorption mode (i.e., ads- or adsorption) by multi-walled carbon nanotubes (MWNT) was investigated. The addition of HS led to a continuous decrease in sorption affinity and an increase in sorption linearity with increasing HS addition. Furthermore, the comparison of distribution coefficients of n- and cycloalkanes showed that the dominant sorption mode remains to be adsorption regardless of the presence of HS on MWNT surface [3]. [1] Wang et al. (2009) Environ.Sci.Technol., 43, 6214-6219. [2] Zhang et al. (2010) Water Research, 44, 2067-2067. [3] Hüffer et al. (2015) Chemosphere 2015, 109, 1169-1175.

**TH207**

Activated Kraft Lignin as an alternative sorbent for sediment remediation with thin-layer capping: Comparison with activated carbon derived from charcoal and coconut

A. Gustafsson, J. Gunnarsson, Stockholm University / Department of Ecology Environment and Plant Sciences DEEP; G. Cornelissen, NGE; E. Sjöholm, Invenema

Thin-layer capping with activated carbon (AC) is a promising remediation technique of contaminated sediments in situ, especially in larger areas, where dredging is not an option. Most carbonaceous sorbents tested for capping are produced either from charcoal or from coconut shells in Asian or African countries and need to be transported long distances for remediation in western countries. There is a need for locally produced sorbents. In this study a new type of C sorbent, derived from locally produced Kraft Lignin (KL), a by-product from pulp production, was compared for its sorption capacity to ACs from charcoal and coconut. Soft wood Kraft Lignin (KL) was isolated from black liquor through a recent biofinery process called LignoBoost, developed to convert lignin into novel biomaterials. The KL was then activated through KOH treatment and pyrolysis. The activated KL (KL-AC) was then compared to AC derived from charcoal (AC-ch) and AC derived from coconut (AC-co) using sorption and bioavailability assays. Sorption assays were done by mixing contaminated sediment (5 g dw) collected from Bergen harbor with 0.5 g dw of activated carbon (AC-Ch, AC-Co or AC-KL) in 40 ml filtered seawater in Erlen flasks. Passive samplers (POM-55) were added, and the flasks were incubated for 72 days on a rotating shaker. After incubation the POMs were extracted and analyzed for PCBs and PAHs. Sorption capacity was comparable for the three AC types with the highest sorption obtained for AC-coconut, followed by AC-charcoal, and then the carbonized lignin (KL). Enrichment factors in the distilled samples were reduced by 92% with AC-ch, 99% with AC-Co and 76% with AC-KL relative to controls without AC. These results were confirmed by the bioavailability assays, DFEs, though often close to the detection levels, showed the same trend as the sorption values. Our results show that activated Kraft lignin could offer a local carbon source alternative to other ACs, should it be produced in sufficient large amount and at a competitive price.

**TH208**

Reduction of copper bioavailability with biochar and compost in vineyard soils with elevated copper concentrations

OMW application (14 L m⁻²) was conducted either in winter, spring or summer on two replicate plots. An addition application was conducted on two irrigated plots in summer. For each of the treatments, we implemented two control plots with tap water application. Soil samples were collected after 2 days, 3 weeks, 6 weeks, 3 months, 6 months, 9 months, 12 months, and 18 months in 5 depth down to 35 cm. Aqueous soil extracts were analyzed for pH, EC, cations, anions and total soluble phenolic compounds. SWR was measured in the field using water drop penetration time (WDPT). Generally, OMW was found to induce SWR. SWR (re-developed gradually during the hot summer season following OMW application. The extent of hydrophobation was strongest in the summer dry, intermediate in the spring and weakest in the most summer application plots. Acidification effects were strongest for summer dry, intermediate for summer wet and lower for spring applications, whereas an effect were observed following winter OMW application. In summer dry application phenolic concentration was higher compared to spring application. During the first rain season following OMW applications, water repellency in all treatments disappeared and the phenolic concentration decreased to the control level. The results indicate that during warm and moist conditions, i.e., when microbial activity is high, phenolic compounds are degraded and the induced compounds probably polymerized thus increasing acidification. Induced SWR was resistant and even increased. Under cold and moist conditions, high initial WDPT rather indicates limitation in hydraulic conductivity than repellency. Phenolic compounds probably were washed out. Keywords: Olive mill wastewater, Water drop penetration time, Acidity, Total phenol

TH211 Does a soil recover within twelve months after olive mill wastewater land spreading? - Searching for a sustainable solution.
M.P. Kurtz, Environmental and Soil Chemistry / Environmental Science; Y. Laor, Agricultural Research Organization / Newe Yaar Research Center RamatVishay Israel; A. Dag, Agricultural Research Organization / Gilat Research Center / Israel; G.E. Schuamann, University of Koblenz-Landau / Institute of Environmental Sciences

Controlled land spreading of olive mill wastewater (OMW) is a widely used agricultural disposal option for this problematic waste. As a product with high nutrient and organic matter content it can serve as fertilizer. However, potential soil hydrophobicity, accumulation of salts and toxic residues like phenols creates problematic side effects. Soil recovery between successive OMW spreading is an important criterion in terms of environmental protection and sustainability when searching for a cheap and effective OMW solution. A field study served to investigate rate dependent soil recovery after two years of consecutive spreading of OMW at rates of 50, 100, 150 m⁻² ha⁻¹ year⁻¹ and, additionally, 100 m³ ha⁻¹ year⁻¹ followed by tillage. The field is an irrigated olive orchard in the semi-arid south of Israel and its soil type is a light brown sandy loam. pH, EC, soluble ions, soluble phenolic compounds (SPC), organic matter, water drop penetration time, soil biological activity (bait-lamina test) were determined depth dependently (0-3 cm, 3-10 cm, 10-20 cm, 20-40 cm, 40-60 cm) twelve months after the last OMW application. Changes of soil properties following OMW application are still detectable 12 months after application of small rates of OMW down to 50 m³ ha⁻¹ year⁻¹. While the upper soil layer showed significant changes compared to the control, which strongly depend on the rate, no effects were found in soil layers below 20 cm. EC and chloride as well as phosphate, organic matter and SPC contents increased significantly in all treatments. Nitrate contents decreased after OMW application. The biological activity in the soils was significantly higher in the treated plots independent of the application rate. Tillage after OMW application decreased the SPC contents compared to the same application rate without tillage and showed also higher biological activity in deeper soil layers. Moreover it had the lowest water drop penetration. Successive and consecutive land spreading of OMW is not supported by the findings of this study. Incomplete degradation of phenolic substances and soil salinization are alarming outcomes with respect to long-term OMW spreading. Tillage after OMW spreading is a promising option towards a sustainable solution.
Advances and criticalities of sub-individual approaches in ecological risk assessment: the way forward (PC)

MOPC01
Consideration of inter-population variability to improve ecological relevance of biomarkers
O. Dedouge-Geffard, University of Reims Champagne-Ardenne / UMRI Unité Stress Environnementaux et BISOsurveillance des milieux aquatiques; a. chaumont, Irstea / UR MALY Laboratoire Ecotoxicologie; A. Jaffal, University of Reims Champagne-Ardenne / UMRI Unité Stress Environnementaux et BISOsurveillance des milieux aquatiques; l. charon; M. Cosin Ponce, University of Reims Champagne-Ardenne / UMRI Unité Stress Environnementaux et BISOsurveillance des milieux aquatiques; A. Geffard, Université de Reims Champagne Ardenne / Interactions Animal-Environnement (IAE); H. Queau, A. Francoix, Irstea centre de Lyon Villeurbanne / UR MALY Laboratoire Ecotoxicologie; O. Geffard, Irstea / UR MALY Laboratoire Ecotoxicologie

Among freshwater species intensively used in ecotoxicology, *Gammarus fossarum* is a relevant test species. In addition to its sensitivity towards contaminants, this species is a common shredder which plays a major role in leaf litter breakdown processes. Many sub-individual and individual responses have been proposed in gammarids in order to monitor and predict effects of chemical compounds on water quality. In this framework, energetic metabolism variations appear to be a good predictive tool to detect physiological disturbance of organisms linked to ecosystem quality. Indeed animal survival depends on the availability of metabolic energy necessary to ensure maintenance, growth and reproduction. Among biological processes to access energy, digestive capacities appear sensitive to contamination stress. In recent studies, based on the use of one reference population, reference and threshold values for digestive enzymes proposed as biomarkers were defined. In the same way, we described link between digestive changes and reproductive impairments. So digestive capacities appear as potential ecologically relevant endpoints to provide information on the consequences likely to occur at the highest hierarchical levels of organization (population). However the use of only one experimental population is not representative of biological diversity. In this way, it appears necessary to precise the between-populations variability of these enzyme activities. Among more than 40 sites investigated, 8 populations of *Gammarus fossarum* considered as reference were selected. On these populations a double approach was applied. In a first step, the basal level of digestive enzyme activities were studied after an acclimation of organisms to similar laboratory conditions. In a second step, the pattern of digestive capacities modulation in the 8 populations exposed at a similar stress (trrophic stress) were considered. This second step aims to identify a potential difference of sensitivity between the eight populations. The results of this preliminary study, underlying changes in digestive enzymes activities, will be discussed according to environmental parameters which could be implicated (different sources of food according to the site) but also concerning the population specific consequences. Reference and threshold values define previously via one control population. Keywords: *Gammarus fossarum*, digestive enzymes, inter-populations variability

MOPC02
Natural variability of some biomarkers in the macro-zoobenthos of streams: dependence and variability on life stage and environmental factors
M. Vighi, University of Milano / Earth and Environmental Sciences; R. Giacchini, University of MilanoBicocca / Department of Environmental and Earth Sciences DISAT; P. Parenti, University of Milano Bicocca / Earth and Environmental Sciences; L. Scarduelli, University of Milano Bicocca / Earth Environmental Sciences

One of the major problems for the use of biomarkers in ecotoxicology is understanding if the measured responses are determined by the effects of stress factors or lie within the natural variability range produced by the effect of environmental parameters. In a previous work, the natural variability of enzymatic levels, measured in some freshwater invertebrate taxa sampled in pristine rivers, was proved to be relevant across both space and time. The results of this work proved that great care should be paid when interpreting experiments in which biomarkers levels are measured and compared among sites or dates; further research is needed to understand how the natural variability of biomarkers could be accounted for and managed in ecotoxicological studies.

MOPC03
Off-site impacts of wildfires - sub-lethal biomarker responses of Gambusia holbrooki to pyrolytic contamination
V. Silva, Universidade de Aveiro / Department of Environment and Planning and the Centre for Environmental and Marine Studies CESAM; I. Campos, Universidade de Aveiro / Department of Environment; B. Nunes, CESAM University of Aveiro; J.L. Pereira, University of Aveiro / Department of Biology and CESAM; P. Pereira, Department of Biology - CESAM; I. Keizer, Department of Environment Centre for Environmental and Marine Studies CESAM University of Aveiro Aveiro Portugal; F. Goncalves, University of Aveiro CESAM / Department of Biology; N. Abrantes, University of Aveiro / CESAMDAO

In the last few decades, the number of wildfires has markedly increased in Mediterranean Europe, including in Portugal. Given both the nature of forest activities, and the fire-propitious meteorological conditions due to climate change, it is expected that the number of occurrences and the burnt area in these countries will further increase. Wildfires have immediate and serious social, economic and environmental consequences. Besides all the direct effects of wildfires, it is recognized that wildfires can be a source of contaminants to downstream water bodies. In fact, pyrolytic substances, such as polycyclic aromatic hydrocarbons (PAHs) and metal and metalloids concentrations), to verify any kind of dependence. Data were elaborated using multivariate statistical methods. In this framework, energetic metabolism variations appear to be a good predictive tool to detect physiological disturbance of organisms linked to ecosystem quality. In this framework, energetic metabolism variations appear to be a good predictive tool to detect physiological disturbance of organisms linked to ecosystem quality. In this framework, energetic metabolism variations appear to be a good predictive tool to detect physiological disturbance of organisms linked to ecosystem quality. The experimental design considered: sampling sites (4 sites in each river) and the addition of some more environmental parameters. The work aimed at identify a potential difference of sensitivity between the eight populations. The results of this work proved that great care should be paid when interpreting experiments in which biomarkers levels are measured and compared among sites or dates; further research is needed to understand how the natural variability of biomarkers could be accounted for and managed in ecotoxicological studies.

MOPC04
From waterborne contaminants to subcellular biomarkers, tissue bioaccumulation, and mussel health: Tracking the effects of waterborne metals throughout the biological hierarchy
A. de Souza Machado, Leibniz Institute of Freshwater Ecology and Inland Fisheries / Ecophysiology and Aquaculture; S.M. Wood, University of Toronto / Department of Biological and Environmental Sciences; A. Bianchini, Universidade Federal do Rio Grande - FURG / Instituto de Ciências Biológicas; P.L. Gillis, Environment Canada / Aquatic Contaminants Research Division

One of the key knowledge gaps in ecotoxicology is the linkage between exposure to harmful contaminants and the cascade of effects that occur through the biological hierarchy. Thus, the present study investigated the relationships between water chemistry (water composition and waterborne metals), subcellular biomarkers (oxidative stress and detoxification biomolecules), an index of biomarker response (IBR), tissue bioaccumulation biomarkers (gill metal and major ion concentrations), and organism general health biomarkers (gonadosomatic index and condition factor) in wild freshwater mussels from Grand River (ON, Canada). Correlation revealed that water chemistry (physico-chemistry and metal concentrations) was the main factor driving all levels of biological responses analyzed (biomolecules to whole organism), explaining 86% to ~100% of variability. Also, a correlation was found between waterborne metals and gill metal content (r=0.92, N=256). Even though water chemistry and biological responses were highly correlated, the levels of individual inorganic contaminants did not exceed Water Quality Guidelines for aquatic life. Thus, biological damage would not be anticipated based solely on water chemistry (i.e. individual inorganic contaminants). However, chronic exposure to the complex contaminant mixture appears to have resulted in several effects. Subcellular and general health biomarkers detected environmental contamination, with variability 55-67% explained by changes in gill metal bioaccumulation, though levels of organic contaminants were not examined. However, IBR did not agree with the environmental metal contamination or urban proximity. Also, subcellular and general health biomarker responses integrated the various environmental stressors and were not significantly correlated. While biochemical biomarkers reflected the exposure gradient of urban-related contaminants, general health biomarkers better

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reflected waterborne metal levels. This examination of multiple parameters and endpoints (e.g. water chemistry, tissue and organism biomarkers) provides insight into the relationships among the observed biological effects and the threat that poor water quality poses to these sentinel organisms. Finally, we propose an alternative index combining water physio-chemistry, waterborne metals and biomarkers to integrate environmental health in a broad scope, and to link water chemistry to responses at different levels of biological organization.

MOPC05 Are transcriptomic biomarkers an added value for sediment-contact bioassays with higher plants? A. Brinke, German Federal Institute of Hydrology; G. Reifferscheid, Biochemistry and Ecotoxicology; R. Klein, Trier University; U. Feiler, Federal Institute of Hydrology; S. Buchinger, Federal Institute of Hydrology / Department G Biochemistry Ecotoxicology

In sediment-contact bioassays with higher plants usuallysediment-bound pollutant induced changes are assessed on a macroscopic scale. As for all biotests the chemical cause for effects remains unknown. Moreover, low concentrations of contaminants can often not be detected. This situation raises the question if classical test systems can be worthwhile enhanced by transcriptomic biomarkers to bridge the knowledge gap between cause and effects, and thus link results from analytical chemistry and ecotoxicological bioassays, as well as to detect small concentrations of bioavailable contaminants. To assess dose-dependent changes, a test protocol for a sediment-contact bioassay with rice was developed. By means of DNA-Microarrays, candidate transcriptomic biomarkers for sediment-contaminations with cadmium and arsenic were identified. Obtained data were verified by means of qPCR. Rice plants were exposed on artificial-sediments at arsenic concentrations from 5 to 15 mg kg\(^{-1}\) and cadmium concentrations from 1 to 400 mg kg\(^{-1}\). Inhibition of root and shoot elongation were assessed as classical macroscopic toxicity endpoints. Root growth proved more sensitive than shoots. EC\(_{50}\) values were assessed as As 13 mg kg\(^{-1}\) / Cd 408 mg kg\(^{-1}\) for roots and 24 mg kg\(^{-1}\) / Cd 477 mg kg\(^{-1}\) for shoots. By means of DNA-Microarray analysis with RNA from root tissue, a subset of transcripts was identified as candidate transcriptomic biomarkers. Hence, root samples (3 time independent exposures with 4 biologically independent replicates each) were exposed at concentrations around and below the EC\(_{50}\). As 11/15 mg kg\(^{-1}\) / Cd 30/400 mg kg\(^{-1}\) to assess effects at low and high concentrations of sediment compounds. Gene expression profiles of exposures were compared to those of references by means of three statistical methods (SAM; LIMMA; T-Test). The list of candidate biomarkers differed between the three statistical methods which were used for data evaluation. In order to define biomarkers which are not dependent from the underlying statistics only genes (n) occurring in all three lists are finally suggested for further studies.

Arsenic induced measurable dose-dependent changes on the macroscopic and the transcriptomic level. Cadmium induced only low dose-dependent changes on the macroscopic level, while clear changes on the transcriptomic level were assessed.

MOPC06 Consequences of adaptation of gammarids to chronic metallic exposures: alterations of biological functions J.D. Lebrun, Irstea; L.C. Fechner, Irstea Antony / UR HBAN; N. Urien, Irstea; I. Friedel, Irstea Antony; E. Gismondi, Laboratory of Animal Ecology and Ecotoxicology; J. Thome, Liege University / Laboratory of Animal Ecology et Ecotoxicology

One of the major issues in ecotoxicology is to link the exposure of aquatic organisms to biological responses. To date, the practical usefulness of biomarkers is limited because of their development in short-time exposure conditions and often, their transient response. Indeed, organisms are generally able to adapt to low environmental contaminations. However, adaptation processes generate additional energetic costs to counteract damages of toxins, at the expense of other biological functions. Thus, characterizing changes in biological traits caused by chronic exposures at the individual level offers interesting alternatives for risk assessment. Because of their ubiquity and ecological roles in freshwaters, gammarids are suitable candidates for such ecotoxicological studies. This study aims at assessing the consequences of adaptation of Gammarus pulex to chronic metallic exposures. During a 3-month period, artificially adapted Gammarus pulex were exposed to environmentally realistic concentrations of Cd, Pb or Cu (0.25, 5 or 1.25 mg/L respectively) in aquatic microcosms. To investigate the cost of adaptation on some biological functions, different traits were monitored, i.e. respiration, locomotion, feeding rate and digestive activities, and compared at the beginning and end of long-term exposures between naïve and artificially-adapted organisms. Results show that respiratory and locomotion were still impacted in gammarids exposed to Cu for 3 months in the same extent as naïve organisms, likely due a persistent toxicity of Cu. Inversely, inhibitive effects of Cd and Pb on these traits disappeared at the end of exposures, suggesting an acquisition of tolerance for these metals. For all tested metals, chronic exposures of gammarids led to a decrease in their feeding rate associated with changes in the composition of ingested leaves. Concerning digestive activities, no effect of Cd was observed in artificially-adapted gammarids. By contrast, \(\beta\)-glucosidase and N-acetyl-\(\beta\)-glucosaminidase activities were specifically inhibited by Pb and Cu respectively, and \(\beta\)-galactosidase activity was inhibited by both metals. To conclude, the adaptation of G. pulex to chronic metallic pressures has a cost for individuals, resulting in alterations of biological functions.

Besides, obtaining biological responses, common to metals or specific to a considered metal, supports the development of biomarkers that could make evidence for long-term impacts of metals on the health of organisms.

Fish model species in environmental toxicology (PC)

MOPC07 Molecular mechanisms of Retinoic Acid isomers in zebrafish eleutheroembryos E. Gismondi, I.T.E.A.-CSC / Environmental Chemistry; M. Casado, Environmental Chemistry; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; C. Barata, CSC / Environmental Chemistry; B. Pina, I.T.E.A.-CSC / Environmental Chemistry

The zebrafish is a established alternative test model for developmental toxicity testing. The normal vertebrate development requires appropriate amounts of Retinoic Acid (RA). All-trans retinoic acid (tRA) and 9-cis retinoic acid (9cRA) are potant metabolites of Vitamin A and operate by affecting gene expression through the retinoic acid metabolic pathway. tRA or 9cRA can bind to retinoic acid receptors (RARs) and the 9cRA isomer bind to retinoid X receptors (RXRs) with more affinity. The exogenous application of retinoic isomers to zebrafish is considered to have deleterious effects of varying magnitudes through Fish developmental stages. In this study, we report both phenotypic and transcriptomic effects of water and fish effluents from exposed RA isomers for 24h and 72h. Nevertheless at sub-lethal concentrations the both RA isomers showed a differential regulation for 3623 features (2-Fold, p<0.01). The transcriptomic analyses reveal profiles describing different patterns, which allow individualizing the action of both isomers through the time. No difference in isomer stability was detected during the period of exposure in embryo water. We concluded that the 9cRA response appears to be deactivated upon time, probably related to either a specific intracellular processing of the molecule or to a genetic negative feedback, and that the presence of any compound masking or anyway interfering with their effective levels may cause harmful effects to developing vertebrate embryos. In conclusion, our data suggests that retinoid effects on early embryonic zebrafish development are not all equally related to the neural-crest system and/or its development. Also, the regulation of the Vitamin A dietary intake may be a special concern for future studies after the development of zebrafish eleutheroembryos.

MOPC08 Genotoxicity assessment of environmental samples under the influence of textile discharges in Salmonella/microsome assay and Fpg-modified comet assay F.J. Vacch, Faculty of Pharmaceutical Sciences; J.A. Vendemiatti, University of Campinas; V. Brosselin, Université de Lyon / ENTP; S. Bony, Université de Lyon; A. Devaux, Université de Lyon-ENPE; G.d. Umbreizu, FACULTY OF TECHNOLOGY -UNICAMP / LEAL.

Surface water and effluents from the influence of textile discharges can exhibit mutagenic activity. In ecotoxicology, in vitro genotoxicity testing often relies on prokaryotic organisms such as in the well standardized Salmonella/microsome assay (Ames test). Many studies have demonstrated that primary DNA damage measurement with the comet assay represents a very early and sensitive genotoxicity biomarker in aquatic species. For in vitro studies, fish cell lines are considered an interesting eukaryotic model retaining specific bio-physiological characteristics. The aim of this study was to measure the genotoxic responses of environmental samples under the influence of textile discharges in Salmonella/microsome assay and in Fpg-modified comet assay. Samples were collected in Piracicaba River upstream and downstream the discharge of Carioba Wastewater Treatment Plant treated effluent and in Quilombo River at Americana city, São Paulo State, Brazil. Organic compounds were extracted from samples by liquid-liquid using dichloromethane and methanol. Salmonella/microsome assays were done using TA98 and YG1041, with and without exogenous metabolic activation (S9). The Fpg-modified comet assays were done after a 24h-exposure of the liver fish cell line RTL-W1. In the Ames assay, higher mutagenicity was detected in strain YG1041 than in TA98, especially with S9, except for Piracicaba River upstream, which was more mutagenic without S9 in YG1041. The genotoxic potential measured by the comet assay was in the same range with and without the enzyme Fpg, except for Piracicaba River upstream, indicating in this case the prevalence of oxidative DNA lesions. Mutagenicity of Piracicaba River upstream was lower than the other sites and presented a different profile indicating the presence of compounds that require S9 to become mutagenic. After the discharges, Piracicaba River becomes more mutagenic and the genotoxic profiles were similar among them and different from upstream. Comet assay was more sensitive than the Ames test to evaluate the genotoxic potential of the samples but these two tests are measuring different types of DNA damage, so they should be considered as complementary.

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MOPC09 Using the zebrafish embryo model to assess potential health effects associated
with indirect potable reuse water
E. Lawton, Brunel University / Institute for the Environment; P. Antczak, University of Liverpool / Institute of Integrative Biology; R. Evans, K. Subramanian, Brunel University / Institute for the Environment; F. Falciani, University of Liverpool / Functional Genomics; S. Johbing, Institute for the Environment; E.J. Routledge, Brunel University / Institute for the Environment

The world’s growing population is causing an ever increasing demand for clean domestic water. In some countries suitable sources of drinking water are becoming scarce and will not be able to satisfy future demand. Consequently, there is a need to find alternative sources of water that can be used for potable supply or to augment current sources. Advanced water treatment methods are now being examined to investigate whether treated domestic sewage effluent can be treated to drinking water standards and discharged near a drinking water abstraction point; a process known as Indirect Potable Reuse (IPR). The aim of this project was to investigate health effects associated with developmental exposure to IPR water at the various stages of treatment using zebrafish embryos. Embryos reared in water at different stages of the treatment process were observed for developmental abnormalities, and differences in gene expression (compared to an aquarium water control) were used to establish both the nature and persistence of these effects along the treatment process. In addition to the embryo assays, passive sampling devices, Pharmaceutical Polar Organic Integrative Sampler (Pharm-POCIS) were deployed over eight, four week periods to collect composite concentrated samples of some of the contaminants present in the effluent. These concentrated extracts were then used in vitro assays; the yeast estrogen screen (as a measure of estrogenicity in the water samples) and an ELISA (to measure the expression of a number of enzymes involved in xenobiotic metabolism) as well as observations of some embryos devoid of pigment. The extracts from less treated effluent inhibited prostaglandin production and had estrogenic activity, however following reverse osmosis both prostaglandin inhibition and estrogenicity were greatly reduced.

MOPC10

Viable compound distribution in zebrafish embryos/larva: the effect of lipophilicity
C. Crumlin, R. Berthelot, Radboud University Nijmegen / University Medical Centre; M. Beekhuisjen, WIL Research Europe BV / Toxobiology; M. Tobor-Kaplon, WIL Research Europe B.V. / GET; B. van de Waart, WIL Research Europe BV / In vitro and Environmental Toxicology; H. Emmen, WIL Research Europe BV / Toxicology

Zebrafish embryos are considered a new promising alternative animal model for developmental toxicity studies. Their organogenesis is of mesodermal origin (Stemple et al., 2003) and this is perceived as a transparent, and this model is not regulated by current legislations on animal welfare up to 120 hours post fertilization (hpf). It is now proposed to be included in the OECD guideline on reproduction toxicity. The predictability of the zebrafish embryo model, however, is highly influenced by compound uptake and thus internal exposure of the embryo and larva. Therefore, the advantages of using zebrafish embryos as a model for global gene expression analysis and detection of NOAEL are highly dependent on the compound distribution within the zebrafish embryo. The effect of lipophilicity was investigated for a total of seven dyes with a wide range of logP values, from which 4,6-Dimethylaminoazobenzene (logP: -4.63), Cresyl fast violet (logP: 3.5), and Sudan III (logP: 7.5) are selected for this study. The use of dyes made the compound uptake and distribution within the embryo and larva visible to the eye. Each compound was tested in forty zebrafish embryos, which originated from two different batches and multiple parents. The exposure period was 96 hours, starting at 4 hpf. Every 24 hours pictures were taken for the evaluation of compound uptake. The pictures taken at 24 hours of exposure to Schiff’s reagent pointed towards gene activity or the yolk sac, while the purple colour within the embryos exposed to Cresyl fast violet is apparent. The most apparent colouration of embryos is observed after exposure to Sudan III, which shows a red glow within the whole embryo and larva. From these observations it can be concluded that higher lipophilicity results in higher compound uptake by the zebrafish embryo.

MOPC11

Identifying genes in the oxidative stress cascade activated through the Nrf2-Keap1 / electrophile response element in zebrafish
A. Foreman, D. Studholme, University of Exeter; T. Kudoh, University of Exeter / Biosciences College of Life and Environmental Sciences; C.R. Tyler, Biosciences College of Life and Environmental Sciences

Oxidative stress is a common toxicological response to chemical and material insult with the production of reactive oxygen species leading to toxic effects induced at both DNA and protein levels. In response to oxidative stress, antioxidant genes are up-regulated though the Nrf2-Keap1 pathway that is highly conserved across animal phyla. Under electrophilic insult, the transcription factor Nrf2 is released from Keap1 and binds to a cis-acting electrophile response element (EpRE) to initiate gene transcription. EpRE sequences have been identified in mammalian species, but little is known currently about functional EpRE sequences in fish. In this study, a bioinformatics approach was used to identify the EpRE in promoter regions of both human and zebrafish genes. A Hidden Markov Model (HMM) was created based on the mammalian EpRE positional weight matrix (PWM) obtained from the JASPAR database and this was searched against human and zebrafish genomes. For the human genome search, the system accurately predicted the functional EpRE sequences for a selection of genes that have previously been identified through experimental methods. A known functional EpRE region in zebrafish Glutathione-s-transferase Pi (GSTpi) was then used as a threshold score and all genes in the zebrafish genome with a score exceeding this were identified and analysed further. Using DAVID, biological pathway analysis was conducted to compare overrepresented pathways from the two outputs and this identified ion regulation to be over-expressed in both the human and zebrafish EpRE containing genes. As metal ions have the potential to produce free radicals, the regulation of ions is essential to prevent an oxidative stress response. Subsequently, the zebrafish gene list was filtered and compared with results generated from next generation sequencing for exposures of zebrafish embryos to silver nanoparticle, ion and bulk materials known to exert toxicity through an oxidative stress response. Identified genes included GSTpi, Glutathione-cysteine-ligase modifying subunit, Heme-oxygenase 1 and Ferritin heavy-chain 1, all recognized targets of Nrf2 in mammals and involved in antioxidant defence. Whole-mount in-situ hybridisation in zebrafish embryos is now being undertaken with these identified genes in order to confirm regulation by Nrf2 through exposure to different inducers of oxidative stress.

MOPC12

Xenobiotic metabolism in zebrafish embryos: enzyme activity in early life stages
B. Schultz, Ecotoxicology; J.C. Otte, BASF SE; D. Vogel, E. Fabian, BASF SE / Experimental Toxicology and Ecology; E. Salinas, BASF SE

Early life stages of the zebrafish (Danio rerio, zf) are increasingly used as model organisms for drug discovery, early teratology screening and chemical testing strategies in (eco-) toxicological assessment (e.g. OECD TG 236; 210; 234). The applicability domain for zf embryos is still expanding due to the many advantages of this biological model (for example fast, external, and translucent development) as well as increasing concern for animal welfare. However, for a well-balanced understanding and interpretation of a (toxicity-) study outcome, it is essential to have knowledge about the potential and extent of xenobiotic metabolism in the system. Current literature on the early metabolism of several xenobiotic metabolizing enzymes in very early developmental stages, however, evidence of enzyme activity is lacking in most cases. In this work the activities of representative phase I and phase II metabolizing enzymes (glutathione-S-transferase, esterase, alcohol dehydrogenase, aldehyde dehydrogenase, N-acetyl transferase 1) were monitored by model substrate transformation using photometric and high pressure liquid chromatography (HPLC) techniques. Six different stages of zf development (between 2.5 hours post fertilization (hpf) to 120 hpf), were investigated. Two main questions were addressed: first, do zf early life stages demonstrate xenobiotic metabolism in a relevant manner for toxicity testing? And second, does this enzyme activity significantly differ during ongoing development? Activities above the methodological level of detection were demonstrated for the enzymes glutathione-S-transferase, reactive intermediates, reductase and N-acyl transferase 1. The results suggested that enzyme activities in zebrafish embryos are discussed. Overall, zf embryos do possess measurable xenobiotic metabolism in early life stages.

Biodegradation and Environmental Fate of Chemicals - Regulatory Acceptance of Non-Standard Tests (PC)

MOPC13

Biodegradation studies of a series of novel 1-Phenylalanine derived Ionic Liquids by a modified Closed Bottle Test protocol including targeted synthesis of persistent metabolites
A.E. Jordan, Chemical Sciences; K. Kümmerr, University of Leuphana / Sustainable Chemistry and Material Resources; A. Hafii, Leuphana University Lüneburg / Institute for Sustainable Chemistry and Environmental Chemistry; J. Westphal, Leuphana University Lüneburg / Technology and Management; M. Spiller, K. Charles University / Centre for New Antivirals and Antineoplastics, Department of Inorganic and Organic Chemistry, Faculty of Pharmacy; D. Coleman, M. Ghavre, Dublin City University; N. Gathergood, Dublin City University / School of Chemical Sciences

The association of Ionic Liquids (ILs) with green chemistry has increased substantially over the past decade concurrent with the upsurge in their use in industrial and wet lab applications and the production of ILs such as the butyl-methylimidazolidium class, have shown to be recalcitrant to biodegradation, calling for the need to examine ILs for biodegradability. The biodegradability of Ionic Liquids has become increasingly more important as a point of characterisation
of ILs. The biodegradation information obtained allows an insight to be gained into how an IL will interact with the environment should it ever enter the environment. The information gained from biodegradation studies also allows for rational design of performance chemicals and should help prevent the synthesis of persistent organic pollutants - the "Benign by Design" concept. Examining ILs for biodegradation is also reinforced by the 10th principle of green chemistry - Design for Degradation. A biodegradation number can be measured by a number of standardized test methods including those supported by the Organisation of Economic Co-Operation and Development (OECD) and the International Organisation for Standardisation (ISO). Problems that exist in the application of these test methods include that a chemical can be classified as readily biodegradable yet still persist as an environmentally persistent metabolite fragment. Modifications to the test methods allow results to be obtained that were not initially accommodated for. In this presentation the results of the biodegradation screening of a series of L-Phenylalanine derived ILs will be presented. Only halide ILs have been screened so as to remove the variability of biodegradable anions. A modified CBT has been employed to analyse persistent chemical fragments that do not readily biodegrade and that are present at the end of the 28 day biodegradation screening. Comprehensive analysis by LC/MS of the residues remaining in the CBT vessel post testing to elucidate the structures of persistent metabolite fragments was conducted. Not all ILs tested gave a persistent fragment and these examples are of paramount importance for designing next generation ILs as they present a platform upon which readily/ultimately biodegradable ILs can be designed from. Avoiding persistent cation/anion fragments as determined by metabolite studies and employing the identified readily biodegradable building blocks presents a innovation in IL design.

MOPC14 Investigation into the biodegradability of various hydrocarbon solvents
C. Hughes, Shell International Limited / Shell Health; G.F. Whale, Shell Health; C. Ouwens, Shell Oil and Gas
Hydrocarbon solvents are petrochemicals used in a wide range of industrial and consumer applications globally. These products consist of many different hydrocarbon constituents and are described as UVVC substances. They are most commonly derived from the processing of crude oil, but other variaties, such as synthetic 'isoalkanes' and solvents derived by the Fischer-Tropsch, or 'Gas-to-Liquid' (GTL) process, also exist. Although different solvents may share similar performance properties and be used in similar applications, their composition varies depending on the origins of the substance. In this presentation studies have been undertaken to assess whether compositional changes are reflected in the biodegradability of solvents. The OECD 301 A-F series of tests are currently used to assess the biodegrability of solvents and the results of these tests are used extensively both within a regulatory context and to support marketing claims on product biodegradability. However, they have their limitations, particularly when they are used to assess the biodegradation of complex and/or poorly water soluble substances. Previous investigations into the biodegradability of GTL fuel and base oil have highlighted these issues. The 301F method is acknowledged as better suited for testing GTL products than 301B. Analysis of vessels at the start and the end of the test indicated that a significant amount of material was lost by abiotic means in the 301B test. This was attributed to the aeration stripping the test substance from the water. The 301F test was found to limit such losses. Based on these findings, the testing of GTL solvents followed a similar methodology. GTL solvents consist predominately of n-alkanes and simply branched isomeric alkanes, so they would be particularly difficult to biodegrade based on the generally accepted view that hydrocarbon classes can be ranked in the following order of biodegradability: n-alkanes > branched alkanes > low-molecular-weight aromatics > cyclic alkanes. For comparison purposes a series of tests was conducted, including hydrocarbon solvents derived by other means. The results of these studies are used to draw conclusions about the biodegradability of hydrocarbon solvents relative to one another. In addition, the challenges associated with measuring biodegradability using the currently accepted methods are further illuminated.

MOPC15 Removal of pharmaceutical wastewater containing erythromycin under anaerobic conditions
Z. Cetecioğlu, Istanbul Technical University / Environmental Engineering; B. Ince, Bogazici University / Institute of Environmental Sciences; M. Gürsoy, B. Rodriguez-Muñoz, Institute for Water Research (ICRA) / Water Quality; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research; D. Orhon, D. Ince, Istanbul Technical University
In this study, the chronic effect and biodegradability characteristics of erythromycin were assessed under anaerobic conditions. For this purpose a lab-scale anaerobic sequencing batch reactor was operated in a sequence of different phases with gradually increasing erythromycin doses of 1.65 – 8.5 mg/L, for more than five months and conventional parameters such as COD, VFA, methane generation were monitored with antibiotic concentrations in wastewater and sludge. Also methanogenic activity of the sludge was determined during experiments. The results revealed that anaerobic treatment is suitable for pharmaceutical industry wastewater up to 8.5 mg/L of erythromycin concentration. 8.5 mg/L and more than this concentration has a terminal/lethal effect on the microbial community under anaerobic conditions, which caused the inhibition of substrate/COD utilization and biogas generation and lead to a total collapse of the reactor. The adverse long-term impact was quite variable for fermenting heterotrophic and methanogenic fractions of the microbial community based on changes inflicted on the composition of remaining/residual organic substrate.

MOPC16 Evidence of enhanced degradation of Prometryn in OECD 307 guideline studies including viable crop root systems
C. Gougoulia, Syngenta Ltd / Product Safety; P. Thomas, CHEFTRA SAS / Ecotoxicology and Risk Assessment; L.H. Hand, Syngenta Limited / Product Metabolism; I.W. Oliver, Keele University / Physical Geographical Sciences; I. Bourke, Syngenta Ltd / Data Management
The environmental fate and behavior of crop protection products (CPPs) in soil is a key part of the regulatory approval process. It is becoming increasingly clear that key contributors to microbial degradation present in the field are not represented in OECD307 guideline studies (i.e. phototrophic soil surface communities and below-ground communities thriving near roots). This may be part of the reason why dissipation rates are often faster in the field than in the laboratory. The purpose of this project was to integrate viable crop-root systems (ryegrass, red clover, hot pepper) into an OECD307 test system to evaluate any differences in the degradation of a model pesticide, compared to the 'bare soil' OECD307 standard. A second control was included to account for any impacts of dynamic temperature or moisture resulting from incubation under a light-dark cycle. Phototrophic soil surface communities were deliberately excluded. All planted and control systems were set up and sampled in triplicate at four time-points after T0. The model compound, 14C-prometryn, was applied to the soil at field rate. Residues were extracted using solvent and quantified by LSC, whilst radioactivity in post-extraction solids, including all plant material, was quantified by combustion and LSC. Volatiles and CO2 were trapped in NaOH to complete the mass balance. Extracts were and analyzed by HPLC with radiodetection to quantify the prometryn concentration. All three chosen crop plants formed and maintained branched root networks of a healthy appearance for at least 63 days of incubation. Compared to the OECD307 control, the time taken for 50% dissipation of prometryn was halved in the systems planted with ryegrass and hot pepper and reduced by a third in the presence of red clover. No significant uptake of radioactivity into the plants was observed. Our work shows that the crop-root networks of different plant groups (monocots, dicots, legumes) are capable of increasing CPP degradation in closed laboratory systems. Further work will be required to investigate the effect on other model compounds and reveal how the microbial communities in laboratory soil systems are altered when crop-root systems are introduced. With the available data, greater environmental realism is conferred by inclusion of crop-plant-root systems, which could be of significance for the environmental risk assessment process.

MOPC17 Comparing laboratory based estimates of dissipation rates for five pharmaceuticals with in situ dissipation rates
J. Klaminder, Ecology and Environmental Science
Pharmaceuticals included in treated wastewater effluent pose a potential threat to aquatic ecosystems, as they may affect non-target organisms in recipient waters. Predictions from laboratory experiments suggest that their dissipation in natural aquatic systems may last at a time-scale ranging from days to month. To what extent standardized laboratory methods are able to detect the biodegradability of chemicals, can be used to predict the dissipation of pharmaceuticals in more complex natural ecosystem environments are rarely tested. In this study we conduct a large-scale experiment where we applied five common pharmaceutical contaminants (Diphenhydramine, Oxazepam, Trimethoprim, Diclofenac and Hydroxycone) to two functional aquatic ecosystems, including natural fish and invertebrates. One of the ecosystems was covered with a tarp to completely remove photodegradation processes. The main objective was to measure the dissipation rates of the drugs from the water phase into the food web (fish and invertebrates) or sediments and compare the results with dissipation rates estimated from laboratory assays. Here, we present for the studied pharmaceuticals: 1) in situ dissipation rates; and 2) quantitative estimates of the relative importance of photo- and microbial degradation in natural waters. The results are compared with similar estimates derived from laboratory incubations. Based on our findings, we discuss the ability to predict the dissipation of pharmaceuticals from aquatic systems using laboratory assay estimates.

MOPC18 Microbial biomarkers as indicators of biotransformation rate of isotope-labelled pesticides in soil
K.M. Nowak, RWTH Aachen University / Institute for Environmental Research Biology; V. A. Mitner; S. Andreas, RWTH Aachen University / Institute for Environmental Research; M. Kästner, Helmholtz centre for environmental research - UFZ / Department of Environmental Biotechnology
Pesticides as anthropogenic chemicals are of high interest; since they are deliberately applied in high amounts to soils to support food crop production. Chemicals entering this complex soil system may undergo various turnover processes. They can be degraded chemically, biologically or microorganisms,
immobilised in the form of non-extractable residues (NER), volatilised or taken up by living organisms. Microbial degradation is considered to be the main pathway of their ultimate detoxification. It is generally known that microorganisms can use C (and N) from an organic contaminant to synthesise their biomass compounds, for instance fatty acids (FA) and amino acids (AA). After death, these biomass compounds can be incorporated into soil organic matter (SOM) forming ultimately biogeochemical cycles. The extraction of known microbial biomarkers from soil after addition of C isotopes allows an estimation of microbial activity in the biogeochemical cycling of C. Microbial biomarker analysis is thus also useful in studying the microbial transformation of labelled pesticides in soil. Therefore, we investigated biotransformation of 2,4-D, glyphosate and bentazon with the particular focus on the metabolic incorporation of the isotope label into FA and AA and their fate in soil over time. 13C-FA and 13C-AA in the living microbial biomass fraction initially increased, thereafter their contents decreased and a continuous incorporation of these biomolecules into the non-living SOM pool was observed. Total amount of the biogenic residues (44% and 21% of 13C-2,4-D and 13C-1,4-N-glyphosate equivalents) and high mineralization rates of both 2,4-D and glyphosate (38% and 75% of 13C-2,4-D and 13C-1,4-n-glyphosate equivalents) suggest that these pesticides were nearly completely biodegraded. However, in the case of more recalcitrant pesticides like bentazon, the incorporation of C into microbial biomass, although reported to be very low, cannot be completely excluded.

Modelling fate & transport of micropollutants: Integration of engineered wastewater systems & natural water bodies - Blind spots & challenges ahead (PC)

MOPC19
Advancements in the assessment of micropollutants through the application of broad-scale “down-the-drain” exposure modelling
K.E. Kapo, R. Vamshi, C.M. Holmes, Waterborne Environmental, Inc.; P.C. De Leo, D. Ferrer, American Cleaning Institute
Municipal effluent is a major exposure route for a wide range of “down-the-drain” chemicals that are treated and discharged to natural water bodies. Exposure models that focus on estimating concentrations of effluent-associated chemicals in receiving waters can serve as a valuable screening-level tool for risk assessment of micropollutants and other potential environmental stressors. In this presentation, we provide an overview of current developments, advancements and challenges in “down-the-drain” exposure modelling, with a focus on iSTREEM®, a web-based model made freely available to the public by the American Cleaning Institute (www.iSTREEM.org). iSTREEM® provides a means to estimate concentrations of “down-the-drain” chemicals in effluent, receiving waters, and drinking water intakes across national and regional scales under mean annual and low (7Q10) flow conditions. The development and evolution of the iSTREEM® model reflects recent trends in technical, conceptual and practical aspects of “down-the-drain” exposure modelling to address current challenges and needs, such as assessment over broad geographies, incorporation ofvariability, and geo-referencing of modelling components. The spatial nature of the model, integrating point locations of facilities along a hydrologic network, provides a powerful framework to assess the environmental risk in a spatial context. To enhance transparency and interpretation of the iSTREEM® model, a case study was conducted comparing national distributions of modelled concentrations of a variety of “down-the-drain” consumer product ingredients to available monitoring data at comparable flow conditions. The iSTREEM® model yielded generally conservative exposure estimates, which can be used to conduct a cost-effective distributed exposure assessment for effluents and streams. Recent technical enhancements and the expanding applications of iSTREEM® will be overviewed, as well as characteristics of iSTREEM® in the context of other current “down-the-drain” models. Enhancing understanding, accessibility and collaboration across the modeling landscape provides opportunity to expand and improve upon individual models and increase their acceptance as a decision-support tool.

MOPC20
Towards spatially smart abatement of human pharmaceuticals in surface waters: defining impact of sewage treatment plants on susceptible functions
L. Coppons, KWR Watercycle Research Institute; A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health
For pharmaceuticals, sewage treatment plants (STPs) are a major point of entry to surface waters. The receiving waters provide vital functions. Modeling the impact of STPs on susceptible functions of the surface water system allows for a spatially smart implementation of abatement options at or in the service area of STPs. This study was performed on a national wide scale for the Netherlands. 31 and 9 rivers from neighboring countries were included as emission points and 2,511 surface water units (SWUs) represent physical parts of the Dutch surface water network. Modeling was performed for two extreme discharge conditions. Monitoring data of 7 locations along the Rhine and the Meuse fall mostly within the range of modeled concentrations. Half of the abstracted volume of raw water for drinking water production, and a quarter of the Natura 2000 areas hosted by the surface waters, are influenced by STPs at low discharge. The vast majority of the total impact via all Dutch STPs during both discharge conditions can be attributed to only 19% of the STPs with regard to the drinking water function, and 39% with regard to the Natura 2000 function. In conclusion, attributing water treatment technologies to STPs as one of the possible measures to improve water quality and protect susceptible functions can be done in a spatially smart and cost-effective way, using consumption-based detailed hydrological modeling.

MOPC21
Modelling and Monte Carlo sensitivity analysis of 8 pharmaceuticals’ occurrence in the Llobregat River (NE Spain)
Z. Banjac, CSIC / IDAEA Environmental Chemistry; A. Ginebreda, CSIC - Spanish National Research Council / Department of Environmental Sciences; R. Marcé, Catalan Institute for Water Research (ICRA); I. Llobregat Water Resources; L. Boitíachs, Catalan Institute for Water Research ICRA; D. Barceló, IQAB-CSIC / Institute for Environmental Assessment and Water Research
Modelling and Monte Carlo sensitivity analysis of 8 pharmaceuticals’ occurrence in the Llobregat river Zoran Banjac, Antoni Ginebreda, Rafael Marcé, Laurie Boitíachs, David Barceló
Modeling the impact of iSTREEM® on the fate of pharmaceuticals in rivers and wastewater treatment plants (WWTPs) presents a multi-parametric problem. For pharmaceuticals, sewage treatment plants (STPs) and receiving freshwater ecosystems to assist decision-making. Microbiological assessment is an essential tool for determining the occurrence of some representative pharmaceuticals (Diclofenac, Ibuprofen, Ketoprofen, Naproxen, Azithromycin, Acetaminophen, Diltiazem and Valsartan) in the Llobregat basin and to assess its sensitivity with respect to the parameters used. The model was constructed on the basis of the steady-state equations describing the fate of the pharmaceuticals in rivers and wastewater treatment plants (WWTPs) with the aid of programming which was used for more efficient Monte Carlo uncertainty analysis. Simulation outputs are statistical distributions of predicted environmental concentrations (PECs) for each river stretch. In order to vary the model results were compared with the experimental data from the project SCARCE. On the other hand we have estimated the sensitivity of the parameters used in the model by calculation of the corresponding sensitivity coefficients defined as CV(PEC)/CV(X) where CV are the coefficients of variation, X the parameters drug consumption. Parameters are: (M), waste water plant removal (Rwwtp), linear velocity of the river (v), river flow (Q), hydraulic residence time (HRT), in stream removal (k) and predicted environmental concentrations (PECs). In order to get more precise estimation the sensitivity analysis is conducted for all stretches of the river. Predicted environmental concentrations are in good order of magnitude with reported measured concentrations(ng/L). Sensitivity analysis shows that removal in WWTP is the most sensitive parameter in the modeling of pharmacokinetics fate in rivers and wastewater treatment plants. Reference: T. Barceló, J. Poch, B. Ochando, et al. (2012). “Occurrence and modeling of pharmaceuticals on a sewage-impacted Mediterranean river and their dynamics under different hydrological conditions.” Science of The Total Environment 440(0): 3-13.

MOPC22
Sources of uncertainty in modelling pharmaceuticals at the basin scale: an inventory across engineered and natural systems
R. Marcé, Catalan Institute for Water Research (ICRA) / Resources and Ecosystems; L. Boitíachs, Catalan Institute for Water Research ICRA; I. Llobregat, ICRA; B. Sch. G. Barrabérint, Catalan Institute for Water Research ICRA; A. Ginebreda, CSIC - Spanish National Research Council / Department of Environmental Sciences; M. Ancochea, RIE; M. Petrovic, ICRA; D. Barceló, IQAB-CSIC / Institute for Environmental Assessment and Water Research; s. sabater, Universitat de Girona
During the last years, increasing interest has gone into chemical fate and transport models for the assessment of chemical concentrations throughout WWTP and into receiving freshwater ecosystems to assist decision-making. These models allow interpreting measured data, extrapolate to new situations, and evaluate proposed solutions for pollution issues. However, the modelling micro-pollutant research community faces a fundamental challenge: the wide range of chemicals to be considered and the emergent nature of most of them imply that few data are available to feed the models. As a result, a high degree of uncertainty is still associated with model estimation and prediction, resulting from the increased knowledge gained from the modelling exercise and the potential management decisions based on the model results. Here, we assess the main sources of uncertainty in the modelling of micro-pollutants concentration across engineered-natural coupled systems, using a humanized basin in NE Spain as a paradigmatic case study. The Llobregat River Basin includes more than 40 WWTPs that discharge in the river network, which is part of the Mediterranean mosaic comprising river reaches from near pristine to highly impacted states. We built a parsimonious model aimed to predict the concentration of 10 pharmaceuticals of human use along the river network considering the following processes: 1) the apparent human consumption of the pharmaceuticals and the load received by WWTPs; 2) the removal of pharmaceuticals in the WWTPs and the load from the effluents to the river; and 3) the apparent in-stream removal during transport along the river network. We assessed the uncertainty of the predicted river concentration arising from the different terms of the model considering the most likely distribution of the values for the different parameters considered in each modelled process. Those distributions were built by mining literature and databases, inverse modelling
approaches, and formulating new empirical relationships for the removal of pollutants in WWTPs with varying technology. The results showed that while the modelling approach seems a suitable tool to advance science and management with the information already available, relevant research topics must be addressed in the following years to reduce the uncertainty of the outcomes. In particular, the uncertainty analysis pointed to the fate of pollutants in WWTP as one of the main sources of model ambiguity.

Impact of statistical methods used in ecotoxicological studies on regulatory decisions (PC)

MOPC25

Comparison of Models for Analyzing Ecotoxicity Studies
C. Teel, DuPont / Applied Statistics; J.W. Green, DuPont / Applied Statistics Group

Several alternative statistical models for analysis of mollusc fecundity, fish early life stage mortality and size, and non-target plant emergence and growth will be compared using a collection of statistical tools appropriate for modeling many ecotoxicity studies. For mollusc fecundity, gamma-Poisson Bayesian models with several alternative underlying concentration-response shapes are compared to one another as well as to several non-Bayesian models with normal error structure. For quantal data (ELS mortality and seedling emergence), quantal regression models with Poisson or negative binomial error structure are compared to probit and non-linear regression models based on proportions. For continuous responses (e.g., size, growth rate), non-linear models with alternative concentration-response shapes and different ways to capture heterogeneous variances are contrasted. Comparisons are made with datasets from guideline laboratory studies and from simulations where the true ECx values are known. General methods for comparing approaches to modeling of ecotoxicity studies are provided and assessed for utility. The relationship between experimental design and model choice will also be explored. The possibility that no suitable regression model can be found will be quantified along with alternative approaches to handle such data.

MOPC26

Ecotoxicology is not normal!
E. Szesz, Institute for Environmental Sciences; R.B. Schaefer, University Koblenz Landau / Institute for Environmental Sciences

Ecotoxicologists perform various kinds of experiments yielding to different types of data often with very small sample sizes and usually not normally distributed. Such data are in most cases analysed with traditional data analysis methods based on the normal distribution. Therefore, they try to transform their data to meet these assumptions and if this fails they refer to non-parametric approaches. Generalized Linear Models (GLM) can fit various types of distributions to data, making a transformation obsolete. However, GLMs are rarely used in ecotoxicology. We simulated two types of data: counts and proportions mimicking data that ecotoxicologists often encounter. We compared models assuming a normal distribution after data transformations (log(Ax +1) for count data and arcsine square root for binomial data) with GLM of appropriate distributions in terms of statistical power and Type-I error. We tested for effects using Likelihood-Ratio-Tests (LRT), F tests parametric bootstrap of these models and a non-parametric approach (Kruskal-Wallis-Test). For extreme sample sizes (N = 3) and count data we found that normal and negative binomial models showed similar performances. However, with increasing samples sizes GLM showed higher power. LRT did not perform well for low sample sizes and parametric bootstrap maintained an appropriate error level. We note, that in our simulation setup (50% reduction in abundance due to treatment) the power was very low for N = 3 (< 50% for models with appropriate error level), which is a common sample size in mesocosm experiments. For binomial data the binomial GLM showed the highest power when sample sizes were extremely low. However, the differences vanished quickly with increasing sample sizes. The non-parametric Kruskal-Wallis test showed low power and was very conservative, especially with small sample sizes. Low replicated experiments are common in ecotoxicology. The power when analysing these should not be further constrained through the use of models not fitting to the data. GLMs provide equal or better statistical properties then models based on data transformations and fit to various types of data without the need of transformations. Our results may also extend to the more arbitrary, where recent GLMs for multivariate analysis have been proposed as alternative to ordination methods. Ecotoxicologist should include GLMs into their statistical toolbox and update the respective statistical guidelines.

MOPC27

Data Should NOT Be Normalized to the Control for Analysis
J.W. Green, DuPont / Applied Statistics Group

A technique unique in the analysis of some ecotoxicity studies is to normalize data in treatment groups to the control. In the case of quantal data (e.g., mortality, emergence, hatching), the method for doing this is sometimes referred to as Abbott’s formula. The original purpose of this method was to adjust mortality data for background incidence. When this idea was first proposed in 1925, most statistical analysis was done manually, so there was a need for simple numerical calculation methods. It has also been used for continuous responses, where the object is to estimate the concentration at which a specified percent effect relative to the control mean response occurs. In this situation, there is no need to adjust for background and the primary purpose in normalizing to the control is to modify the data so that a probit analysis (which assumes quantal data) software package can be used. Better ways to take background mortality into account or model continuous responses have been known for several decades and software is widely available to carry out these alternative methods. It will be proved not only is the analysis of normalized data theoretically unsound and unnecessary, but is misleading. In the context of several types of guideline studies, it will be shown that ECx estimates obtained from analysis of normalized data (actual or continuous) can over- or underestimate the true value by a large factor when other readily available approaches provide accurate estimates.

MOPC28

Considerations in selecting endpoints from ecotoxicity tests: An example using algae and cyanobacteria
J.P. Staveley, Exponent

Regulatory authorities are increasingly looking to make decisions based on an EC5 or EC10 endpoint, perhaps due to wide-spread criticisms of the NOEC. However, it is important to recognize the types of data suitable for ECx approaches, in particular understanding which tests, rather than an arbitrary assumption, are best for use in the selected response parameter. In the U.S., harmonized test guidelines for pesticides and industrial chemicals for toxicity testing with algae and cyanobacteria include the EC50 and the NOEC as test endpoints, and state that if the NOEC cannot be determined, the EC50 is used instead. A meta-analysis of toxicity test data for algae and cyanobacteria was conducted to examine the inherent variability in the test design and the validity of a 5% effect measure. Three species of microalgae and one species of cyanobacteria were included in the analysis. The coefficient of variation (CV) and standard error (SE) were calculated for the following growth parameters at 96 hours: yield, area under the growth curve (AUGC), and growth rate. The analysis indicated that the CV varies depending on the growth parameter and is often greater than 5%. For growth rate, the control CV can be expected to be 3%–7%, while for yield and AUGC, it can be expected to be 10%–20%. For cyanobacteria, these values are even greater. The measure of variability is reduced when based on SE, which accounts for the number of replicates, but is still not consistently below 5%. There are conceptual, statistical, and risk assessment reasons why the EC50 is not an appropriate endpoint for toxicity tests with algae and cyanobacteria. Concerning the statistical reasons, the results from this analysis indicate that it is not possible to reliably distinguish the effects of test substances from the control on growth responses of algae at a 5% level, and this level is even greater for cyanobacteria. The analysis confirms the contention that the appropriate value of the ECx for use in risk assessment will vary depending on the test species, measurement parameter, and test design. The path away from use of the NOEC will require a similar evaluation of the suitability of types of ecotoxicity tests, rather than an arbitrary assumption that the EC50 (or other ECx) is suitable in its place. The use of the ECx requires a decision on what value of “x” is both biologically and statistically significant.

MOPC29

Environmental risk assessment of diclofenac: roots of uncertainty
J. Garrig, Institut National de Recherche pour l’Agriculture, l’Alimentation et l’Environnement (INRAE)

In 2013, diclofenac was added to the watch list for emerging pollutants under the Water Framework Directive in order to gather sufficient information on its occurrence in European rivers. Indeed, concern arose from studies showing adverse effects in trout tissues at the low µg/L. Therefore, we reviewed 24 studies that assessed diclofenac chronic effects on freshwater species (classical and biomarker endpoints) and we proposed a quantal risk assessment method that includes the quotient method (ratio PEC/PNEC). The PEC was calculated following the guideline for ERA of medicinal products for human use, whereas the PNEC was derived following the Technical Guidance Document (TGD) of ECHA. Our work aims at assessing the impact on risk assessment outcome of (i) the method used to derive the PNEC (species sensitivity distribution, SSD, or assessment factor, AF) and (ii) choice of the dataset (with or without biomarker results), in order to illustrate current hurdles in ERA procedures. Considering all data, the ERA outcome depends on the method used to derive the PNEC. The RQ is above 1 (RQ=7.5) with the PNECSS, whereas it is below 1 (RQ=0.5 [0.04;3.7]) with PNECF, although showing a huge confident interval (CI) which includes 1. In contrast, without biomarker data and in RQ=0.5 [0.04;3.7] with the PNECSS, the risk assessment method used. Biomarkers orientate clearly the regulatory decision for diclofenac. According to ECHA TGD, biomarkers should be “toxicologically relevant in term of population dynamics”. However, only 2/7 studies investigated concomitantly biomarkers and classical endpoints. Some biomarkers lacked of specificity (e.g. oxidative stress) and displayed no clear dose-dependent effect. Furthermore, we identified discrepancies in protocol design regarding histology findings (4/7 studies, e.g. grade of severity), impeding direct comparison of results. Overall, our analysis illustrates known issues related to ERA procedures such as the influence on regulatory decision of the method for PNEC derivation and the choice of dataset. The SSD provides a CI and seems more robust, whereas the use of AF is more protective and can be applied in case of few data. Biomarkers have attractive features, however, this case study highlights a need for a quality assurance for measurement of biomarker (e.g. standard protocol) and for biological linkage with higher level changes (e.g. using a suite of endpoints) in order to promote their use for regulatory purposes.
MOPC30
How to obtain robust data from aquatic higher tier studies: lessons learned
P. Lopez-Manciscidor, Dow Agro Sciences LLC / Ecotoxicology; G. Meregalli, Dow AgroSciences Italia srl / Regulatory Sce & Government Aff.; N. Polletka, Dow Agrosiences / RSGACES; I. Barber, Dow Agrosciences
The aim of this study was to re-evaluate 5 micro-/mesocosm studies in order to assess the reliability of the results to be used for the derivation of RACs based on the ecological threshold option (ETO-RAC) and/or the ecological recovery option (ERO-RAC) to be used during the registration procedure for pesticides in the EU. The Minimum Detectable Differences (MDD) calculation was selected as the statistical tool to evaluate the reliability of the endpoints derived from these studies following the recommendations of the new EFSA Aquatic Guidance Document (AMR 2007). The results and conclusions derived from this re-evaluation will be used to make recommendations for the design of further micro-/mesocosm experiments. The results obtained in the re-evaluation of the results obtained from the different micro-/mesocosm studies showed an acceptable robustness in some of them, however for some taxa (especially for some macroinvertebrates) the MDDs values are above 100% and no clear treatment related effects could be determined because of the low power of the analysis. For one of the insecticides, the NOEC derived for Chaoborus spp. differs between the three studies available and the calculated MDDs values can be used to determine which is the most reliable NOEC to be used in the risk assessment. It can be concluded that special attention should be taken during the pretreatment period to try to avoid the variability amongst the replicates that can affect the robustness of the data obtained. Other factors that should be taken into account during the design of the experiment is the sampling methodology for macroinvertebrates and emerged insects to try to optimise the number of individuals sampled. These conclusions are in line with those stated in Brock et al. (2014) and can determine the reliability of these studies for the derivation of the Regulatory Acceptable Concentrations.

MOPC31
Impact of fracking on ambient PAH levels in a rural community
L. Giudice, C.E. Donald, B. Smith, Oregon State University / Environmental and Molecular Toxicology; L.G. Widtow, Oregon State University / Environmental Molecular Toxicology; K.A. Hobbie, Oregon State University / Environmental Molecular and Toxicology; L. Kincl, Oregon State University / College of Public Health and Human Sciences; E. Haynes, University of Cincinnati College of Medicine / Department of Environmental Health; K.A. Anderson, Oregon State University / Environmental Molecular Toxicology
Unconventional natural gas drilling, or “fracking,” has increased rapidly in the U.S. in recent years. Coupled with a lack of regulation, this has raised concern about environmental and health impacts. To address this, passive air samplers were deployed in a rural Ohio community that has been heavily impacted by fracking. Trained landowners mailed samplers to Oregon State University, where they were analyzed for 62 polycyclic aromatic hydrocarbons (PAHs). Data were grouped based on distance from the sampler to the closest active fracking well. Three distance groups were created, with the “close” group < 0.1 from an active well, the “middle” group from 0.1 to 1.0 mile from an active well, and the “far” group >1.0 mile from an active well. Average PAH levels increased as samplers got closer to active wells. This is indicative of what is measured in samples from the close group were predominantly petrogenic, while PAHs had a stronger pyrogenic signature as samplers moved farther from active wells. Quantitative human health risk assessments estimated the excess lifetime cancer risks (ELCRs) associated with exposure to measured PAH levels. For a maximum residual exposure of 24 hours/day, the ELCR decreased from to 262 to 176 in a million when moving from the closest to far group. All ELCRs were above the level that the U.S. EPA considers acceptable. This work suggests that fracking activity could be contributing significantly to the ambient PAH load, at levels that are relevant to human health.

MOPC32
Identification and assessment of compounds contributing to acute toxicity of Oil Sands process affected water
G. Morandi, University of Saskatchewan / Department of Veterinary and Biomedical Sciences and Toxicology Centre; S.B. Wiseman, University of Saskatchewan / Toxicology Centre; k. zhang, University of Alberta; A. dos Santos Pereira, University of Alberta / Division of Analytical and Environmental Toxicology; R. Martin, Stockholm University / DIV OF ANALYTICAL AND ENVIRONMENTAL TOXICOLOGY; K.M. Werman, UNIVERSITY MEDICINE AND PATHOLOGY; J.P. Giesy, University of Saskatchewan / Toxicology Center
Oil Sands process-affected water (OSPW) produced during the extraction of bitumen in the surface-mining Oil Sands industry in Alberta, Canada, is acutely and chronically toxic to aquatic organisms. It is known that organic compounds that are dissolved in the OSPW are responsible for most toxic effects, but knowledge of the specific chemicals that cause this toxicity, or the associated mechanism(s) of toxicity, is limited. Using a top-down approach, a spreadsheet model has been developed to predict the acute toxicity of the organic fraction of OSPW to early-life stages of fathead minnow (Pimephales promelas). Mixture composition and the concentration of individual mass fractions is characterized by use of linear ion trap (orbitrap) ultrahigh resolution mass spectrometry. Measured octanol- water partition coefficients (log kow) are established by polydimethylsiloxane (PDMS)-coated stir bar sorptive extraction. A narcissus mode of action is assumed and the target-lipid model of Di Toro et al., 2000 is applied for toxicity predictions of individual mass fractions assuming concentration addition of the individual mass fractions. The predicted toxicity of the organic fraction of OSPW was compared to the observed toxicity from in-lab 96-hr assays of embryo lethality. Predictions compared well with measured acute toxicity and highlights the usefulness of this spreadsheet model for predicting the acute toxicity of the organic fraction of OSPW to embryos of Fathead minnow. Using a biosassay for individual analysis, we have measured fractions of OSPW organics which have acute toxicity. Accuracy of this model to predict the toxicity of acutely toxic fractions of OSPW organics was within 45% of the measured values and within the 95% CI of the measured dose-response relationship.

MOPC33
Substitution of hazardous offshore chemicals in UK waters: an evaluation of their use and discharge from 2000 to 2012
M. La Vedrine, Cefas Lowestoft Laboratory / Chemical Assessment Team; D. Sheahan, R. Gioia, B. Rowsles, S. Kroeger, C. Phillips, Cefas; M. Kirby, The offshore oil and gas industry will use and discharge large quantities of substances for the extraction of hydrocarbons. Within the OSPAR Convention, the toxicity of the whole mixture is predicted based on distance from the sampler to the closest active fracking well. Three Trained landowners mailed samplers to Oregon State University, where they were deployed in a rural Ohio community that has been heavily impacted by fracking. Quantitative human health risk assessments estimated the excess lifetime cancer risks (ELCRs) associated with exposure to measured PAH levels. For a maximum residual exposure of 24 hours/day, the ELCR decreased from to 262 to 176 in a million when moving from the closest to far group. All ELCRs were above the level that the U.S. EPA considers acceptable. This work suggests that fracking activity could be contributing significantly to the ambient PAH load, at levels that are relevant to human health.

Scales and interactions in community and ecosystem ecotoxicology (PC)
TUPC01
How food web interactions and toxic stress combine to affect biodiversity
D. Kulkarni, Laboratory of Environmental Ecosystem Ecology; C. Melian, Swiss Federal Institute of Aquatic Science and Technology Eawag / Center for Ecology Evolution and Biogeochemistry; F. De Laender, Université de Namur ASBL / Laboratory of Environmental Ecosystem Ecology
Biodiversity is an important aspect of our community structure and a predictor of ecosystem functioning. Chemicals have the potential to affect biodiversity of key trophic levels such as primary producers and consumers but if and how these effects propagate in ecosystems through food web interactions (top-down and bottom-up processes) is unknown. We extended a recently developed community model, that considers both intra- and interspecific tolerance variability to describe toxic effects on biodiversity the species abundances within a larger metacommunity, to multistrain fractions of OSPW organics which have acute toxicity. Accuracy of this model to predict the toxicity of acutely toxic fractions of OSPW organics was within 45% of the measured values and within the 95% CI of the measured dose-response relationship.
diversity using the ISI web of Science database. A synthesis of observed literature data was compared to model predictions. At lower concentration values, a toxicant-induced reduction in predator diversity led to increased prey diversity while a toxicant-induced reduction in prey diversity led to reduced predator diversity. Effects at higher concentration values were less pronounced compared to those at lower concentration values. Top-down and bottom-up cascading effects of predator and prey diversity have been documented in scientific literature. While a decrease in prey diversity led to an increase in stressors, the decrease in prey diversity is shown to have both positive and negative effects. Our model can explain the top-down effects of predator diversity as well as the negative bottom-up effects of prey diversity. We conclude that food web interactions under toxic stress significantly contribute to changes in plankton biodiversity and these changes are more pronounced at low concentration values.

TUPC02
Effects of intra- and interspecific competition on the sensitivity of Daphnia magna populations to the fungicide carbendazim
A. Del Acro, UNIVERSITY OF JAEN / ANIMAL BIOLOGY PLANT BIOLOGY AND ECOLOGY; P. van den Brink, Alterra/Wageningen UR / Aquatic Ecosystem and Water Quality Management Group b Alterra; A. Rico, Wageningen University / Environmental Risk Assessment

The Ecological Risk Assessment (ERA) of pesticides is based on information obtained from single-species toxicity tests in which ecological interactions (i.e., competition, predation) are not taken into account. This poses a challenge for the extrapolation to real cases where ecological interactions are given weights. It is expected that competition (intra- and interspecific) will result in additional stress, thus resulting in an increase of the sensitivity of aquatic populations to toxicants. In order to test this hypothesis, an experiment with five levels of competition (i.e., control, medium and high intraspecific competition, medium and high interspecific competition) and four carbendazim (i.e., control, low, medium and high exposure concentration) treatments was set up. The observed impacts of competition on the population dynamics of the crustacean Daphnia magna under different competition levels. The competition was established based on algal food availability, using different D. magna densities for the intraspecific competition, and Brachionus calyciflorus as competing species for the interspecific competition. The experiment was performed in glass jars (1.5 L volume) under controlled laboratory conditions, and consisted of a pre-treatment period of 4-days, in which competition was allowed to occur, and a 21-day exposure period. Results of these experiments showed no statistically significant differences owing to combined effects of carbendazim and competition. However, species interactions resulted in noticeable effects when comparing the corresponding D. magna EC50 values for each treatment level, after a stressor exposure period of 14-days. While intraspecific competition slightly increased the sensitivity of D. magna, as it was expected, interspecific competition seemed to reduce it. The later response was most likely related to an indirect effect explained by the alleviation of the food limitation stress caused by the grazing of D. magna over B. calyciflorus. These diverse responses point out the complexity of population responses to environmental changes for ecological interactions are taken into account. In conclusion, competition effects are not easy to classify, but experiments suggest that they play an important role in defining population-level effects of pesticides. Hence, more studies are needed to better characterize ecological interactions before they are included in future ERAs.

TUPC03
Combined effects of temperature and zinc on a microcosm freshwater community
D. Van de Perre, Ghent University Laboratory of Environmental Toxicology and Aquatic Ecology / GhEnToxLab; F. De Laender, Université de Namur ASBL / Laboratory of Environmental Ecosystem Ecology; I. Roessink, Alterra; P.J. van den Brink, Alterra/Wageningen UR / Aquatic Ecosystem and Water Quality Management Group b Alterra; A. Rico, Wageningen University / Environmental Risk Assessment

Combined effects of temperature and zinc on a microcosm freshwater community. An indoor freshwater microcosm experiment was performed in glass jars (1.5 L volume) under different competition levels. The competition was established based on algal food availability, using different D. magna densities for the intraspecific competition, and Brachionus calyciflorus as competing species for the interspecific competition. While intraspecific competition slightly increased the sensitivity of D. magna, as it was expected, interspecific competition seemed to reduce it. The later response was most likely related to an indirect effect explained by the alleviation of the food limitation stress caused by the grazing of D. magna over B. calyciflorus. These diverse responses point out the complexity of population responses to environmental changes for ecological interactions are taken into account. In conclusion, competition effects are not easy to classify, but experiments suggest that they play an important role in defining population-level effects of pesticides. Hence, more studies are needed to better characterize ecological interactions before they are included in future ERAs.

TUPC04
Spatially-explicit individual-based models of competition: impact of stress on resilience
h. baveco, Environmental Risk Assessment Team; A. Focks, Alterra Wageningen University and Research Centre / Ecotoxicology Environmental Risk Assessment Team; F. De Laender, Université de Namur ASBL / Laboratory of Environmental Ecosystem Ecology; M. Tijiau, Institut de Radioprotection et de Sûreté Nucléaire; P.J. van den Brink, Alterra/Wageningen UR / Aquatic Ecosystem and Water Quality Management Group b Alterra; A. Rico, Wageningen University / Environmental Risk Assessment

Predicting and understanding the consequences of global warming on aquatic macrophyte-dominated outdoor mesocosms in two European ecoregions R. Mueller, Goethe-University, Institute of Occupational, Social and Environmental Medicine / Aquatic Organisms and Ecosystems; C. Shinn, IMAR-Marine and Environmental Research Centre / Laboratoire Evolution et Diversité Biologique; M. Moreira-Santos, University of Coimbra, IMAR-CMA / Department of Life Sciences; R. Bieiro, University of Coimbra / Environmental Risk Assessment Team; Acknowledgement – The Chimeras project is financed by the Long Range Initiative of CEFIC (www.cefic-iri.org) (project code: LRI-ECO19)

TUPC05
Long-term effects of the fungicide pyrimethanil on aquatic primary producers in macrophyte-dominated outdoor mesocosms in two European ecoregions

R. Mueller, Goethe-University, Institute of Occupational, Social and Environmental Medicine / Aquatic Organisms and Ecosystems; C. Shinn, IMAR-Marine and Environmental Research Centre / Laboratoire Evolution et Diversité Biologique; M. Moreira-Santos, University of Coimbra, IMAR-CMA / Department of Life Sciences; R. Bieiro, University of Coimbra / Environmental Risk Assessment Team; Acknowledgement – The Chimeras project is financed by the Long Range Initiative of CEFIC (www.cefic-iri.org) (project code: LRI-ECO19)

TUPC06

Rational design of chemicals and pharmaceuticals and other input prevention measures towards sustainable technological development (PC)

TUPC07
How environmentally friendly are cholinium based ILs?
A.M. Gonçalves, Biology; J. Santos, University of Porto, CIMAR & ICBAS, Laboratory of Ecotoxicology; J. Pereira, University of Aveiro & CESAM / Department of Biology; F.L. Silva, S. Ventura, J.L. Coutinho, University of Aveiro / Chemistry; F. Goncalves, University of Aveiro CESAM / Department of Biology

Ionic liquids (ILs) are salts with low melting points and liquid at or close to room temperature. Their cation group designates the IL family and both the cation and the anion can be functionalized depending on the intended properties. Their rational (environmentally concerned) design can benefit from information retrieved by the ecotoxicological analysis of the ILs, since the effects of the functionalized headgroups, there is an increasing number of cholinium-based ILs being synthesized, but this family is not well characterized yet. This interest is based on the fact that cholinium is an essential nutrient, so these ILs are believed to be solvents with lower environmental hazardous potential, with a multitude of biotechnological applications. In order to properly evaluate the ecotoxicity of this IL family, a range of aquatic and terrestrial organisms (including fungi and bacteria) and with structures that allow for further functionalization.

TUPC08
Atom Economy, Catalysis and Green Toxicology: Tools for Sustainable Chemistry and Drug Discovery
N. Gathergood, Dublin City University / School of Chemical Sciences, Sustainable chemistry research projects combine three fields of green chemistry; ionic liquids, ecotoxicity and catalysis. One study is directed towards ionic liquids which can also catalyse reactions. This overlap between organocatalysis and ionic liquid research enables us to design low toxicity and potentially biodegradable catalysts based on the extensive biological screening database of ionic compounds. The performance of a new catalyst, in tandem with the ecotoxicity screening allows the chemist to develop greener synthetic methods.1 We have designed a library of apricot ionic liquids which can act as Bronsted acid catalysts with low antibacterial and antifungal activity. Toxicity evaluation of all the ionic liquids prepared was performed to establish the influence of ester or amide groups in the cation. Antimicrobial and antifungal toxicity results show that the ionic liquids did not inhibit the growth of any organism screened at concentrations of 2.0 mM. Biodegradation of the novel ionic liquids was also investigated in “CO2 Headspace” test (ISO 14593). Our latest work which includes green chemistry metrics feedback, investigates the effect of substitution on the imidazolium ring on biodegradation, toxicity and activity of the catalyst.2 This enables our team to recommend the ‘greener’ catalyst of our series for further applications. The second part of the talk will introduce the concept that drug discovery can be linked to the search for novel sustainable catalysts scaffolds. References 1) Gore, R. G.; Truong, T.-K.-T.; Pour, M.; Myles, L.; Connion, S. J. and Gathergood, N. Green Chem., (2013), 15 (10), 2727-2739. 2) Myles, L.; Gore, R. G.; Gathergood, N. and Connion, S. J. Green Chem., (2013), 15 (10), 2740-2746. 3) Gore, R. G.; Myles, L.; Spuls with a bio-derivative, L. G.; Myles, L.; Connion, S. J. and Gathergood, N. Green Chem., (2013), 15 (10), 2747-2760.

TUPC09
The effect of aromatic groups on the antimicrobial toxicity of ionic liquids derived from phenylalanine and mandelic acid
H. B. França, Dublin City University / School of Chemical Sciences; F. Goncalves, University of Aveiro / Chemistry; M. Gurbisz, M. Ghavre, Dublin City University; F.C. Ferreira, São Paulo State University UNESP; F. Goncalves, University of Aveiro CESAM / Department of Biology; I. Beadham, B. Quilty, Dublin City University; J.L. Coutinho, University of Aveiro / Chemistry; N. Gathergood, Dublin City University / School of Chemical Sciences; D. Colona, Dublin City University; M. Spulak, Charles University / Centre for New Antivirals and Antimicrobials, Department of Inorganic and Organic Chemistry, Faculty of Pharmacy; M. Garcia, Institute of Advanced Chemistry of Catalonia

Ionic liquids (ILs) are often described as ‘green solvents’; mainly due to their very low vapour pressure. However, assessment of their environmental impact is also a recommendation. The ILs must not produce any long-term environmental effects, but could have a limited toxicity to the environment. This is a challenging goal for IL research today. The work of the Gathergood research group is aimed towards the synthesis and analysis of biodegradable, low toxicity ILs and their applications. The Gathergood group has proposed that using renewable resources to form ILs could improve the eco-toxicity of the compounds. Thus, work has been carried out on the synthesis of ILs using bio-derived anions, namely mandelic acid and the amino acid phenylalanine.1 A broad series of ILs using these two bio-renewable resources as chiral scaffolds has been synthesised and their antimicrobial toxicity evaluated. ILs from phenylalanine with various headgroups and aromatic substitutions have been synthesised. Modification of the headgroups on the phenylalanine scaffold has allowed for toxicity studies to be carried out on the effect of aromatic and non-aromatic headgroups. The focus of the testing allowed us to determine the limited toxicity to the environment. This enables our team to recommend the ‘greener’ catalyst of our series for further applications. A broad series of ILs using these two bio-renewable resources as chiral scaffolds has been synthesised and their antimicrobial toxicity evaluated. ILs from phenylalanine with various headgroups and aromatic substitutions have been synthesised. Modification of the headgroups on the phenylalanine scaffold has allowed for toxicity studies to be carried out on the effect of aromatic and non-aromatic headgroups. The focus of the testing allowed us to determine the limited toxicity to the environment. This is a challenging goal for IL research today. The work of the Gathergood research group is aimed towards the synthesis and analysis of biodegradable, low toxicity ILs and their applications. The Gathergood group has proposed that using renewable resources to form ILs could improve the eco-toxicity of the compounds. Thus, work has been carried out on the synthesis of ILs using bio-derived anions, namely mandelic acid and the amino acid phenylalanine.1 A broad series of ILs using these two bio-renewable resources as chiral scaffolds has been synthesised and their antimicrobial toxicity evaluated. ILs from phenylalanine with various headgroups and aromatic substitutions have been synthesised. Modification of the headgroups on the phenylalanine scaffold has allowed for toxicity studies to be carried out on the effect of aromatic and non-aromatic headgroups. The focus of the testing allowed us to determine the limited toxicity to the environment. This enables our team to recommend the ‘greener’ catalyst of our series for further applications. A broad series of ILs using these two bio-renewable resources as chiral scaffolds has been synthesised and their antimicrobial toxicity evaluated. ILs from phenylalanine with various headgroups and aromatic substitutions have been synthesised. Modification of the headgroups on the phenylalanine scaffold has allowed for toxicity studies to be carried out on the effect of aromatic and non-aromatic headgroups. The focus of the testing allowed us to determine the limited toxicity to the environment. This enables our team to recommend the ‘greener’ catalyst of our series for further applications. A broad series of ILs using these two bio-renewable resources as chiral scaffolds has been synthesised and their antimicrobial toxicity evaluated. ILs from phenylalanine with various headgroups and aromatic substitutions have been synthesised. Modification of the headgroups on the phenylalanine scaffold has allowed for toxicity studies to be carried out on the effect of aromatic and non-aromatic headgroups. The focus of the testing allowed us to determine the limited toxicity to the environment. This enables our team to recommend the ‘greener’ catalyst of our series for further applications. A broad series of ILs using these two bio-renewable resources as chiral scaffolds has been synthesised and their antimicrobial toxicity evaluated. ILs from phenylalanine with various headgroups and aromatic substitutions have been synthesised. Modification of the headgroups on the phenylalanine scaffold has allowed for toxicity studies to be carried out on the effect of aromatic and non-aromatic headgroups. The focus of the testing allowed us to determine the limited toxicity to the environment. This enables our team to recommend the ‘greener’ catalyst of our series for further applications.
traditional organic solvents in vast variety of applications. The number of combinations of cations and anions forming ionic liquids (ILs) is practically infinite and they can be custom designed in order to suite the desired application. But the complete information on their environmental impact is not available. It is clearly not feasible to analyze the toxicity of so many compounds. The application of in silico methods such as QSAR could speed up the process of (eco)toxicological evaluation and predict the potential toxicity of ILs before they are considered for commercialization. The QSAR modeling can allow reliable prediction of toxicity, avoiding unnecessary animal experiments, which is also encouraged by ECHA and REACH. In this study, a group contribution QSAR model was used in order to predict the (eco)toxicity of eight protic ILs belonging to a new short aliphatic family which had not been previously experimentally analyzed in five (eco)toxicity test methods: Pseudokirchneriella subcapitata and Lemna minor growth inhibition test, and Acetylcholinesterase inhibition and Cell viability assay with IPC-81 cells. The predicted toxicities confirmed the previous findings on the new family of protic ILs, and these eight representatives also showed low or no toxicity. The only toxic effect can be seen towards Lemna minor in case of two ILs which have EC50-1, and according to the globally Harmonized System of Classification and Labelling of Chemicals can be classified as harmful for the aquatic environment (category Acute 3). Additionally, a QSAR prediction of the EC50 values for other ILs was performed and compared with the experimentally determined EC50 and high correlation coefficients were obtained, thus confirming the accuracy of the model. The in silico QSAR model applied in this study could allow the selection of potentially less toxic ILs amongst the existing ones, but it can also be a basis for changing the chemical structures and properties towards developing new, “greener” ILs, respectful with the environment.

TUPC11
Metabolite analysis of a series of novel L-Phenylalanine derived Ionic Liquids using a modified Closed Bottle Test protocol and high resolution Orbi-trap MS

A. Hais, Leuphana University Lüneburg; J. Westphal, Leuphana University Lüneburg / Technology and Management; A.E. Jordan, Chemical Sciences; N. Gathergood, Dublin City University / School of Chemical Sciences; K. Kramerer, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry

Ionic Liquids (ILs) are increasingly seen as an important building block of green chemistry over the past decade because of their specific properties e.g. as solvents such as the potential for high recyclability, low-volatility, low-flammability, low-toxicity, and the potential for synthesis from renewable resources. New applications are anticipated for ILs that may result the introduction of ILs into the aquatic environment. In another greenhouse gas, to be fulfilled by green and sustainable ILs is fast and full mineralisation after they are introduced into the aquatic environment. However, early generations of ILs have shown to be recalcitrant to biodegradation, calling for the need to examine ILs for biodegradability. Currently, biodegradability can be measured by a number of standardized test methods. These tests do not reveal any detailed information on chemical processes and chemical species involved in biodegradation. A deeper insight into the course of biodegradation of ILs can be gained by employing high resolution Orbi trap MS. This will allow for the rational design of ILs according to the “Benign by Design” concept. The biodegradation screening of a series of L-Phenylalanine derived ILs was performed (28 and 42 days was performed. The fragments detected were then synthesised from first principles and their MS/MS was compared to those extracted from the CBT vessel to complete the structural elucidation. Only halide ILs have been screened so as to remove the variability of biodegradable anions. None of the ILs cations tested reached the ready biodegradability threshold with 28 days. One did after 42 days indicating the adaptation of microorganisms to the test compound. TPs have successfully been identified by high resolution MS. Not all ILs tested gave a persistent fragment. The identification of recalcitrant TPs is gave new insights thus demonstrating the added value of the approach. The results are of paramount importance for designing next generation ILs as they present a platform upon which readily/ultimately biodegradable ILs can be designed from. Avoiding persistent cation/anion fragments as determined by metabolite studies and employing the identified readily biodegradable building blocks presents a innovation in IL design.

Mercury Pollution: Chemistry, Remediation and Policy (PC)

TUPC13
Mercury Regulation Issues in Armenia

A. Aleksandryan, Hazardous Substances & Waste Policy Division / Head of Division

The Republic of Armenia signed Minamata Convention on Mercury that is the main strategy document for regulation of mercury, its compounds with the aim to reduce or to where possible, eliminate mercury use in industrial sectors and supposes to systematically reduce emissions and releases to land and water, and phase out the use of mercury where alternatives exist, as well as recycle wastes. At present, inArmenia mercury is used and emitted from industrial applications (lighting equipment, chlorine production, cement production, mining, synthetic rubber production, and pesticides) and medical appliances (dental amalgam filling and thermometers). The import and/or export of chemicals and pesticides are regulated by the Rotterdam Convention. The List of chemicals and pesticides regulated by Rotterdam Convention and banned in the Republic of Armenia involves compounds of mercury, including: inorganic mercury compounds, compounds of alkyl mercury, as well as alkylalkylmercury and arylated compounds of mercury, which are prohibited by the Governmental Decision. Another important legislative document is the List of wastes containing mercury compounds that was approved by the Governmental Decision. This List includes (i) wastes containing mercury, mercury compounds as a component or pollutant, (ii) scrap (wastes), electrical equipment or electro-technical nodes, involving galvanic elements, batteries, mercury switches, glass of cathode ray tubes and other types of glass with the active covering or polluted by cadmium, mercury, lead, polychlorinated biphenyls at the concentration level from 5 % to 1 % and above, (iii) soft and hard lamps and luminescent tubes. The List of theRepublicofArmenia banned hazardous waste, among others, includes wastes containing mercury, mercury compounds as a component or pollutant; the List was approved by the Governmental Decision. In theRepublicofArmenia the List of wastes banned for import was also approved. All the Decisions and by-laws are of importance for the sound management of mercury, mercury compounds, as well as mercury containing products and wastes.

TUPC14
Bioavailability of mercury: benthic organisms vs. passive sampling

L. Marziali, IRSA-CNR (Brugherio); M. Camusso, Italian National Research Council Water CENIRSA; S. Pascaletti, A. Dragio, IRASCNRC Brugherio; F. Rossignoli, Italian National Research Council Water Research Institute CENIRSA; G. Tartari, IRASCNRC Brugherio / UOS Brugherio; A. Marchetto, CNR ISE; L. Guzzella, Italian National Research Council Water Research Institute CENIRSA

Mercury bioavailability was assessed in the Toce river (North Italy), were residual contamination deriving from the activity of a chlor-alkali plant is present in sediments (values up to 0.05 mg kg⁻¹) and (ii) worked medicinal waters (values up to 3 mg kg⁻¹). Mercury concentrations that grazers, confirming that exposure is mainly due to the ingestion of contaminated food (sediments or prey), By comparison, DGT samplers proved to be effective tools in on-site exposure for assessing Hg bioavailability to benthic organisms. As a confirmation, C. riparius and DGT samplers showed similar response in the lab exposure. The different approaches will be considered for the ecological risk assessment of the riverine ecosystem.

TUPC15
Mercury levels in forest and grassland soils in the surrounding area of a cement plant in NW Spain

L. Cuíllas-Barreiro, University of Vigo; P.P. Rodríguez, Universidade de Vigo / Plant Biology and Soil Science; A. Gómez-Armesto, University of Vigo / Plant Biology and Soil Science; J. Nóvoa-Muñoz, M. Arias-Estévez, University of Vigo; E. Martínez-Rodríguez, X. Fernández-Sanjuán, A. Núñez-Delgado, University of Santiago de Compostela

Cement plants have increased its contribution to emissions of heavy metals into the atmosphere due to the presence of the pollutant in cement raw materials (limestone and clay), as well as to the burning of fossil fuels (coal, fuel, gas). Although control systems are in place to minimize pollution risks, cement factories emit a significant burden of Hg, a volatile heavy metal with high toxicity potential. As a result, Hg is frequently found in soils of the surrounding areas, where it may cause relevant environmental problems. In this study, soil samples were taken in forest and grassland soils, at intervals of T) concentrations were determined using an Hg auto-analyzer. Forest soils showed Hg concentrations between 41 and 117 µg kg⁻¹, being higher in organic than in mineral fractions. By comparison, DGT samplers proved to be effective tools in on-site exposure for assessing Hg bioavailability to benthic organisms. As a confirmation, C. riparius and DGT samplers showed similar response in the lab exposure. The different approaches will be considered for the ecological risk assessment of the riverine ecosystem.

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Various bacterial guilds contribute to methylation mercury formation in sediments contaminated by sewage treatment plant discharges

A.G. G. Bravo, Department of Ecology and Genetics, Limnology / Department of Ecology and Genetics Limnology; J. Zopfi, University of Basel / Environmental Geosciences; S. Bertillon, Uppsala University / Department of Ecology and Genetics Limnology; S. Bouchet, Université de Pau et des Pays de l’Adour / LCABIE/IPREM UMR CNRS; D. Amroux, LCABIE IPREM / UMR CNRS; J. Dominik, Istituto di Scienze Marine - Consiglio Nazionale delle Ricerche; C. Cosio, Geneva University / Institute Forel Earth and Environmental Sciences

The methylation of inorganic-Hg (HgII) to methylmercury (MeHg), which is bio-accumulated and biomagnified in food webs, is a major concern because MeHg can damage human health. The formation of MeHg, (i.e. Hg methylation) takes place under suboxic to anoxic conditions and it is genetically mediated by sulfur-reducing bacteria (SRB), iron-reducing bacteria (FeRB), methanogens and/or firmicutes. Previous studies demonstrated a concomitant release of Hg, iron and organic matter by many sewage treatment plant (STP) discharges. The aim of this study was thus to assess the impact of the STP’s discharges on Hg methylation in sediments, with a particular attention to the role of iron and different bacterial guilds on Hg methylation. We combined a geochemical and microbiological characterization with Hg speciation and sediment amendment experiments to evaluate the impact of STP’s effluents on MeHg production. We collected sediment material at two stations in the Vidy Bay that differ in their degree of exposure to STP emissions. In all samples proteobacteria was the dominant phylum (28.6 ± 1.4%) of total OTU. In all experiments, FeRB, SRB, and the obtained values were better correlated to the cationic exchange capacity of the sediment and plant species as test organisms, and covered a wide range of long-term and short-term lethal and sublethal endpoints. Aquatic tests used leachates from the test plants and the obtained values were better correlated to the cationic exchange capacity (CEC) than the organic matter (OM) content. Surprisingly, no influence of copper was observed on propranolol adsorption in spite of the formation of a Cu(II)-propranolol complex previously evidenced. Thus, propranolol mobility in the environment is not influenced by the presence of copper. Keywords: adsorption, metal, beta-blocker, soil, pharmaceutical

Two methods to reduce cadmium in wheat crops

T.H. Landberg, Stockholm University / Department of Ecological Environmental and Plant Sciences; M. Geger, Stockholm University / Department of Ecology Environment and Plant science

Cadmium (Cd) is a highly toxic and hazardous element. In Sweden 43 % of the daily Cd-intake originates from wheat, due to high Cd accumulation in wheat grains and a large consumption of wheat products. To decrease the Cd intake the Cd uptake by wheat plant from soil and the transport of Cd to the grains should be prevented. The aim was to test two methods to decrease Cd concentration in wheat grain in field cultures: 1) phytoextraction (use of plants to remove Cd from the soil) and 2) silicon (Si) supplementation, where Si make Cd less available for plants and the transport from root to shoot of Cd is prevented by Si. Phytoextraction was performed by the use of Salix (Salix viminalis) cultivated in clayey and sandy soil, respectively, for 1-4 years prior to cultivation of wheat (spring and winter wheat; Triticum aestivum). Wheat was then cultivated several years after the soil has been remediated. Silicon was added (0-500 kg/ha) one month after sowing in spring wheat culture. Results showed that already after one year of phytoextraction the soil Cd concentration decreased with 8%. After 4 years phytoextraction the Cd concentration decreased with up to 25 % and that of wheat grains with up to 32%. When Si was added, the largest decrease of Cd in wheat grains was 10%, found at the highest Si addition. It was found that for a Si effect more than 160 kg/ha of Si has to be added.

Mercury distribution in two soils from Almadén contaminated by sewage treatment plant discharges

J. Bori, Universitat Politècnica de Catalunya (UPC) / Centre for Research and Innovation in Toxicology CRIT; A. Arnaiz, Universitat Politècnica de Catalunya UPC; B. Valles, Universitat Politècnica de Catalunya UPC / Centre for Research and Innovation in Toxicology CRIT; J. Millán, Universitat de Castelló-La Mancha; M. Riva, Universitat Politècnica de Catalunya UPC

Ecosystems in the mining district of Almadén (Ciudad Real, Spain) are one of the most mercury contaminated worldwide, but little is known about the toxic effects on biota. In the present study, potential toxicity of soils collected from Almadén was examined through different terrestrial and aquatic ecotoxicity tests. Soil toxicity testing was performed with Eisenia fetida and Daphnia magna. The obtained results were determined by the adsorbed amounts depend on the soil chemical properties. The adsorption coefficients K_d were determined and the obtained values were better correlated to the cationic exchange capacity (CEC) than the organic matter (OM) content. Surprisingly, no influence of copper was observed on propranolol adsorption in spite of the formation of a Cu(II)-propranolol complex previously evidenced. Thus, propranolol mobility in the environment is not influenced by the presence of copper. Keywords: adsorption, metal, beta-blocker, soil, pharmaceutical

Soil and water pollutants’ assessment, monitoring and remediation (PC)

TUPC19

Co-adsorption of propranolol and copper (II) on soils

R. SMITH, Champagne-Ardenne; S. Sayen, University of Reims Champagne-Ardenne; E. Guillon, Université de Reims Champagne Ardenne / Institut de Chimie Moléculaire de Reims ICMP CNRS Groupe Chimie de Coordination

The methylation of inorganic-Hg (HgII) to methylmercury (MeHg), which is bio-accumulated and biomagnified in food webs, is a major concern because MeHg can damage human health. The formation of MeHg, (i.e. Hg methylation) takes place under suboxic to anoxic conditions and it is genetically mediated by sulfur-reducing bacteria (SRB), iron-reducing bacteria (FeRB), methanogens and/or firmicutes. Previous studies demonstrated a concomitant release of Hg, iron and organic matter by many sewage treatment plant (STP) discharges. The aim of this study was thus to assess the impact of the STP’s discharges on Hg methylation in sediments, with a particular attention to the role of iron and different bacterial guilds on Hg methylation. We combined a geochemical and microbiological characterization with Hg speciation and sediment amendment experiments to evaluate the impact of STP’s effluents on MeHg production. We collected sediment material at two stations in the Vidy Bay that differ in their degree of exposure to STP emissions. In all samples proteobacteria was the dominant phylum (28.6 ± 1.4%) of total OTU. In all experiments, FeRB, SRB, and the obtained values were better correlated to the cationic exchange capacity of the sediment and plant species as test organisms, and covered a wide range of long-term and short-term lethal and sublethal endpoints. Aquatic tests used leachates from the test plants to assess the growth inhibition of Pseudokirchneriella subcapitata and the lethality on Daphnia magna. Results showed that, despite the extremely high concentrations of mercury in some soils, this metal did not cause a significant change in most ecotoxicity tests. This lack of response may be due to mercury speciation in this abandoned mining area. It has been long documented that Hg in Almadén’s soils is mainly found associated to the most resistant forms and, nevertheless, the lowest Hg concentrations are found in more labile forms (water-soluble phases, exchangeable fraction, carbonates and amorphous oxyhydroxides). Anyway, despite of the low percentages of Hg associated to labile forms, the actual contents of easily available Hg are important since Hg contents in the study original soils where mining activities were carried out, are high (from 5 to 40000 mg kg⁻¹). Therefore, the actual content besides percentage should be considered in order to assess potential decontamination strategies and future safe land uses for minimizing human and environmental risks. Keywords: mercury, SEP, mine area, Almadén

Contaminations of industrial soils are generally difficult to assess due to the presence of complex mixture of many pollutants. The identification of contaminants which are responsible for biological effects is an important issue for the remediation of industrial sites and soils. Effect-directed analysis (EDA) is a quite new approach which combines bioassays, chemical fractionation and chemical analysis to identify pollutants having biological effects. The use of EDA to identify contaminants in industrial soils focuses on novel compounds

Effect-directed analysis of Ahr-active organic compounds in industrial soils contaminated by crosseye plant mining residues

M. Delapalouhoue, LPTC EPOC UMR5805 / LPTC EPOC UMR; S. Ait-Aissa, INERIS / Ecotoxicology Unit; H. Budzinski, University of Bordeaux / UMR EPOC Equipe LPTC

Soil and water pollutants’ assessment, monitoring and remediation (PC)
that would allow to adapt soil remediation methodology based on identified molecules and to find new relevant molecules to track for soil risk assessment. The methodology should be fast, cheap and repeatable in order to interest the industries. Here, EDA has been developed with an extraction of pollutants by pressured liquid extraction, and fractionation by normal phase HPLC. It was been applied on an industrial site contaminated by creosote, a complex mixture containing several hundreds of molecules. Then if PAHs are fractionated of its mass. The results show that this soil is contaminated by molecules able to activate the aryl hydrocarbon receptor. The fractionation shows a good repeatability and a separation of the AhR activity among different fractions. AhR activity is distributed between fractions 7 and 16 with a more important activity for fraction 7 and 8. With this fractionation methodology, PAHs are eluted in fraction 9. The AhR activity for fraction 7 and 8 is responsible of 66% of total AhR activity. By using toxic equivalent factors (TEF) based on Benzo(a)pyrene, the 16 PAHs of US-EPA explain 4% and 70% of AhR activity in F7 and F8 respectively. However, majority of AhR activity is due to another molecules that 16 PAHs of US-EPA and which are not quantified for soil risk assessment. Furthermore, majority of these AhR activators are eluted with a more polar fractions involving that molecules are more hydrophilic and possibly more mobile. GC-QToF analyses are under progress to identify molecules present in active fractions.

TUPC22
Impact of mining activities on ecosystems: studies under semiarid Mediterranean climate in SE Spain
J. Álvarez-Rogel, H.M. Conesa, I.M. Párraga-Aguado, M. Tercero, Escuela Técnica Superior de Ingeniería Agronómica Universidad Politécnica de Cartagena / Ciencia y Tecnología Agraria; M. González-Alcaraz, Faculty of Earth and Life Sciences, VU University / Ecology & Society

This presentation is an overview of the results obtained in a number of recent studies carried out in the old mining area of La Unión-Sierra de Cartagena and in the polluted wetlands of the Mar Menor lagoon (SE Spain). The mine soils, and the soils and marine bodies affected by them, showed a wide range of pH values, from acidic (pH 3-4) to neutral-basic (pH 7-8). The whole of the results indicated extremely high concentrations of metals/loid(s) in the tailings, the surrounding areas and the affected wetlands (in mg kg⁻¹): As (~100 to ~2000), Cd (~30 to ~120), Cu (~50 to ~150), Mn (~4000 to ~13000), Pb (~1500 to ~20000) and Zn (~4000 to ~31000). The concentrations of extractable metal/loid(s) were considerably lower and depended on the chemical reagent used (deionised water, EDTA and CaCl₂). Metal accumulation was found in inland and wetland plants and in marine molluscs. In the mine tailings, after the pioneer plants started to colonise small patches of factories, plant communities adapted to the conditions. A facilitation effect of pioneer plants that were able to grow. Late successional stages were found as part of the so-called ‘fertility islands’, showing that natural succession might be successful. In the most extremely saline environments of the polluted wetlands, the disturbance due to mine wastes was not determinant in influencing vegetation composition, but it was important in degraded dune systems and dry watercourses. Greenhouse experiments showed that in the absence of flooding conditions, liming decreased the soluble metal concentrations and favoured the growth of Sarcocornia fruticosa (a halophyte species), and enhanced the capacity of this species to phyto-stabilise metals in the roots. When neutral wastes were subjected to alternating flooding-drying conditions, liming decreased the concentrations of Mn, Zn and Cd in pore water of bare soils, but not Cu and Pb. When liming and plants were combined, S. fruticosa counteracted the effect of the amendment, strongly increasing the concentration of metals in pore water. In acidic wastes (pH ~3.5-4.0), liming decreased the soluble Zn, Cu and Pb concentrations, but increased soluble Mn and Cd, especially in the treatment with liming and S. fruticosa. These studies revealed that the phytomagement of areas affected by mine wastes must consider the specific characteristics of each case and that the use of non-specific remediation measures is strongly advised against.

TUPC23
Activated carbons prepared from plum, apricot and cherry kernels: Influence of operational parameters and elemental composition on the yield of product
S. Cremona, A. Guzman, J. Palacios, S. Kostic, G. Perez, Faculty of Earth and Life Sciences; J. Radonic, Faculty of Technical Sciences; M. Brboric, M.V. Miloradov, Z. Djukic, M.S. Turk, University of Novi Sad / Faculty of Technical Sciences

The effective removal of heavy metals from aqueous wastes is among the most important issues for many industrialized countries. Activated carbon, an adsorbent with large porous surface area, controllable pore structure and thermo-stability is being extensively used in environmental and pollution problems in the wastewater treatment processes (removing of dyes, heavy metals and polyaromatic hydrocarbons). Different kinds of agricultural residues could be utilized and the production of activated carbons for the tertiary treatment of industrial wastewaters would be achieved at a reasonable cost. The use of alternative activated carbon derived from biomass, an agricultural waste material, has been investigated as a replacement for the current expensive methods of removing heavy metals from wastewater. Plum, apricot and cherry kernels, a low-cost biomass residue, were used to prepare activated carbon by thermochemical activation with phosphoric (H₃PO₄) and sulphuric (H₂SO₄) acid as the activating agents at 400°C and 500°C for 2 h. In the study, various parameters such as the impregnation type, activation temperature and elemental composition of kernels were evaluated to establish the yield of product. It was concluded that operational parameters and composition of row materials have significant influence, in the range of 75% to 40%, on the yield of the synthesized activated carbons. Acknowledgement: This research has been financially supported by Ministry of Education, Science and Technological Development, Republic of Serbia (III46009).

TUPC24
Toxicity assessment of soils in a recovered area affected by a mining spill
F.M. Peinado, A. Romero Freire, University of Granada / edadología y quimica agricola; M. SIERRA ARAGON, E. FERNANDEZ ONDONO, University of Granada / Soil Science Department; F.J. Martín Garrizón, University of Granada / edadología y quimica agricola; I. ORTIZ BERNAD, University of Granada / Soil Science Department; O. Kodirov, Academy of Sciences Uzbekistan / Institute of Geology and Geophysic

In 1998 a breach in the Aznalcollar pyrite mine (Sevilla, Spain) spilled around 4.5 x 10⁶ m³ of acidic waters and toxic tailings containing high concentrations of zinc (Zn), copper (Cu), cadmium (Cd), arsenic (As), lead (Pb), and thallium (Tl) into the Agrio and Guadianar river basins. One of the most important soil-rehabilitation programmes in Europe was applied to restore the affected area, concluding with the establishment of the Guadiamar Green Corridor. Although the remediation measures gradually decreased the concentration of pollutants in the affected soils, an irregular distribution pattern of contamination in the affected soils was easily discernible, with highly polluted spots alternating with other much less polluted areas. Fifteen years after the accident, most of the affected soils are recovered, although highly polluted spots of soils with variable area (from 1 to 200 m²) are easily distinguished in the landscape because no vegetation has developed, representing a major source of pollution that should be monitored. The aim of the present work is to assess the residual contamination and potential toxicity of the unrecovered soils affected by the mine spill 15 years after the accident by the use of toxicity bioassays. The residual pollution is distinguished in the field because no vegetation has developed. The analytical characterization of the area indicate that the unrecovered areas have acidic pH, high concentrations of total Pb and As, and high concentrations of soluble Zn, Cu and Cd. The toxicity bioassays with Lactuca sativa and soil respiration indicate no toxic effect in the recovered areas, but significant toxic effect were detected in the bare soils, where the non-carbonate area showed higher toxicity than the carbonate sector. Thus, the unrecovered soils have become major sources of pollution, requiring intervention in order to raise the pH, decrease the solubility of pollutants, and encourage colonization by vegetation.

When organic contaminants interact with particles: consequences on fate, exposure and effects (PC)
toxicity, bioaccumulation, pollutants

TUPC26 Adsorption of phenanthrene to carbon nanotubes: Influence on PAH bioavailability and ecotoxicity A. Boedt, SINTEF Materials and Chemistry / Environmental Technology; B. Grael, F. Zindler, Norwegian University of Science and Technology; D. Alin, Biotrix AS; L. Støen, SINTEF Materials and Chemistry / Environmental Technology

In the present study, adsorption of dissolved phenanthrene to aqueous dispersions of a suite of different CNTs containing dissolved natural organic matter (NOM) was investigated. The study also investigated the influence of the adsorption process on the bioavailability and subsequent ecotoxicity of dissolved phenanthrene to standard freshwater test species representing two different trophic levels; the microalga Pseudokirchneriella subcapitata and the cladoceran water flea Daphnia magna. The dispersion concentration and stability of the different CNTs in different ecotoxicity media was determined with and without NOM present. In addition, the direct ecotoxicity of the CNT suite was investigated with both species at environmentally realistic dispersion concentrations. The influence of CNT surface area and surface chemistry on phenanthrene adsorption and its subsequent ecotoxicity were investigated. Five different CNTs, including one single walled carbon nanotube (SWCNT), two multi walled carbon nanotubes (MWCNT-2 and MWCNT-3) and two functionalised MWCNTs (MWCNT-OH and MWCNT-COOH) were used in the study. Specific surface area and surface chemistry of the CNTs appeared to be important factors in controlling both the fate of CNTs (dispersion concentration and dispersion stability) and their interaction with phenanthrene in aqueous environments. Polar surface functional groups increased the dispersion and stability of CNTs, but reduced their adsorption capacity for phenanthrene. The higher specific surface area of SWCNT compared to the MWCNTs was related to higher phenanthrene adsorption. The CNT having the highest surface area, also exhibited the highest adsorption capacity and the lowest stability in aqueous media over time. A significant change in phenanthrene toxicity to P. subcapitata was only seen in the presence of one type of CNT (SWCNT) when considering total phenanthrene concentration in the system. Based on the measured concentrations of phenanthrene in the water phase, however, an increase in toxicity in the presence of CNTs was observed relative to phenanthrene only. This indicates that not only the phenanthrene remaining in the water, but also the phenanthrene adsorbed to CNTs, is available to the algae. An increased exposure concentration to algae attached to CNT aggregates might explain the increased toxicity observed based on the dissolved phenanthrene concentration. Data for D. magna are currently being generated.

TUPC27 Do nanoparticles affect the toxicity of neonicotinoid insecticides? C.S. Lorenz, Universität Tübingen / Animal Physiological Ecology; L. Jäger, Tübingen University / Animal Physiological Ecology; L. Guluzada, Tübingen University / Center for Applied Geosciences; L. D’Aniello, Tübingen University / Institute of Inorganic Chemistry; Y. Liang, Tübingen University / Institute of Inorganic Chemistry; R. Anwander, Tübingen University / Institute of Inorganic Chemistry; S. Haderlein, University of Tübingen; H. Kohler, University of Tübingen / Animal Physiological Ecology

Artificial nanoscale particles are frequently in use nowadays and have various areas of application. However, little is known about their fate and interactions in the environment. This study is to investigate, how the toxicity of the neonicotinoid insecticides imidacloprid and thiocloprid is altered by nanoparticles that potentially adsorb these insecticides. Effects will be examined on the non-biting midge, Chironomus riparius, a well-established study organism in ecotoxicology, for which the impacts of these neonicotinoid insecticides are already known. Hence, based on the OECD guideline 217E, we examine several endpoints in the life cycle of C. riparius including survival rate, weight gain, emergence rate, and the induction of the stress protein hsp70 as a marker for proteotoxicity. Tests with the neonicotinoid insecticides or nanoparticles as single substances are conducted, followed by experiments with mixtures of them. For the latter experiments, one can hypothesize that nanoparticles enter an organism easily due to their small size, hereby increasing the uptake of insecticides associated to the nanoparticles. Consequently, the nanoparticles would act as ‘Trojan horses’, and the efficiency of the insecticides may be increased. Another possible mixture effect could be that the nanoparticles are largely excluded from uptake by the epithelia of C. riparius. Consequently, less insecticides would be bioavailable to the midges, resulting in weaker toxicity. Moreover, in future experiments, nanoparticles with a fluorescent core will be used which enables to track them in the organisms.

TUPC28 Fullerenes soot modifies the toxicity of organic xenobiotics in ecologically relevant conditions J. Sanchis, M. Olmos, IDAEA CSIC / Water and Soil Quality Research Group; M. Farre, IDAEA CSIC; D. Barcelo, IQAB-CSIC / Institute for Environmental Assessment and Water Research Fullerenes are carbon nanomaterials with relevant scientific interest and potential market applications in fields as diverse as personal-care products, water treatment, electronics and solar cells. Because of their use in nanotechnology and their incidental emission from combustion sources, fullerenes can be found in the environment. This has raised health and environmental concerns and many studies have been recently conducted in order to assess the toxicity of fullerenes to different organisms. Nevertheless, these tests have often been conducted in non-realistic conditions, suspending fullerenes in water with the help of surfactants, ultrasonos or by solvent-transfer method. In the present work, the acute toxicity of fullerene soot has been tested simulating ecologically relevant scenarios. Fullerenes were dispersed by intensive ultrasonic treatment, or by aeration and sunlight exposure, in artificial sea water, artificial estuary water and artificial fresh water, with different concentrations of organic matter. Their toxicity to Vitrbo fischeri and Daphnia magna was assessed by means of standardized tests. Fullerenes toxicity was highly dependent on both, salinity and humic acid concentrations, following the Freundlich equation, and both xenobiotics were slightly different in their reactivity in the bibliography, highlighting the relevance of suspending nanomaterials in environmentally extrapolable conditions. Moreover, the toxicity of binary mixtures fullerenes soot–organic pollutants was assessed with five xenobiotics. In most of the cases, fullerenes aggregates immobilized the organic pollutant because of their sorptive properties and the activity of pesticides was severely inhibited. However, in some particular cases, a synergism between fullerene aggregates and the organic pollutant was observed in Daphnia magna. This situation strongly suggested that fullerenes, when bioaccumulated in daphnids digestive tract, may act as vectors for other pollutants, enhancing their uptake and toxic effects, what is known as Trojan Horse Effect.

TUPC29 Preliminary study on the interaction of Carbon Black nanoparticles and B(0)P on zebrafish embryos C. Della Torre, University of Siena / Department of Physical Earth and Environmental Sciences; L. Del Giacco, University of Milan; N. Santo, Universit degli Studi di Milano; S. Magni, M. Ascgini, A. Ghilardi, L. Prosperi, D. Magni, University of Milan; M. Petrini, University of Milan / Biology; A. Binelli, University of Milan / Department of Biosciences Carbon Black Nanoparticles (CBNs) have been investigated as benchmark control for in vivo toxicological evaluation of diesel exhaust particles and as a model of urban air pollution particulate matter. On the contrary their toxicity for aquatic biota is almost unknown. CBNs are ROS generators and can mediate genotoxicity by means of inflammation and oxidative stress. Moreover CBNs show strong sorption capacity to organic pollutants as PAHs. Therefore in the ternary system organism-pollutant- CBNs two interactions are possible: CBNs might act as carrier of toxic pollutants inside the cell, thus producing synergistic adverse effects. Otherwise CBNs could bind the compound decreasing its bioavailability for biota. The aim of this study is therefore to evaluate the contribution of CBNs as environmental pollutant as B(0)P to CBNs toxicity and vice-versa on zebrafish (Danio rerio) embryos. Firstly, CBNs standard was cleaned-up with toluene in a Soxhlet extractor up to 92 h, evaluating the effective elimination of PAHs impurities by a GC-MS/MS. Results highlighted a strong sorption of PAHs to CBNs standard and the need to purify it before the following exposure tests. The zebrafish embryos were visually assessed for malformation at different times (24, 48, 72, and 96 hours post fertilization) under stereomicroscope, transmitted light microscope and fluorescence microscope. The uptake of CBNs and B(0)P and their intra-cellular or tissue target, as well as the possible damage of the biological structures have been investigated by means of advanced microscopy techniques. Our results highlight that the contribution of the sorbed environmental contaminants to CBNs toxicity represents a key feature in the ecotoxicological studies.

From ecosystem services to risk assessment for aquatic and terrestrial plants (PC)

TUPC31 ECOSYSTEM SERVICES, ENVIRONMENTAL STRESSORS AND DECISION MAKING - RESULTS OF A GLOBAL SETAC AND ESA PELLSTON WORKSHOP J. Van Wensem, TCB; C.S. Duke, Ecological Society of America; E. Huber-Sanwald, Instituto Potosino de Investigacion Cientifica y Tecnologica (IPICYT); L. Kapustka, LK Consultancy; L. Malthy, The University of Sheffield / Dpt of Animal Plant Sciences; D.W. Moore, ENVIRON International Corp.; W.R. Mortensen, US EPA / Athens, Division

There is increasing awareness that improved environmental management can be achieved by considering more explicitly the benefits that humans receive from ecosystems. In a broad sense, the contributions of ecological systems to the health
and well-being of people can be considered ecosystem goods and services (hereafter ecosystem services). The ecosystem service concept provides a framework for considering whole ecosystems in decision making, for valuing the services they provide, and for ensuring that society can maintain a healthy and resilient natural environment now and for future generations. A Pellston Workshop was convened by SETAC and the Ecological Society of America (ESA) in Shepherdstown, WV, USA, at the end of September 2014 to develop: 1) broadened and updated, and provision of the ecosystem services concept to environmental decision making as part of a movement towards environmental sustainability; and 2) work products in the form of scientific manuscripts, booklets, and presentation materials needed to promote environmental stewardship through application of the ecosystem services concept by the memberships of SETAC and ESA, and society more generally. Thirty participants from academia, government, industry, and NGOs around the world worked toward consensus on a path forward for putting the ecosystem services concept to practical use in decision making. Individual workgroups considered five main topics: 1) ecosystem services, protection goals and environmental decision making; 2) understanding and applying ecological production functions; 3) applying the ecosystem services concept to risk assessment; 4) applying the ecosystem services concept in natural resource management and restoration; and 5) practical guidance for applying the ecosystem services concept in environmental decision making. This presentation highlights the key findings and recommendations of each workgroup, together with overall recommendations for improving the use of ecosystem services in decision making.

TUPC32 Modelling the impact of herbicide exposure on the structure and composition of non-target terrestrial plant communities J. Rees, K. Bergholz, University of Potsdam; T. Schad, EnSA; K. Körner, University of Potsdam; A. Solga, Ecology; T.G. Preuss, Bayer CropScience / Environmental Modelling; F. Jeltsch, University of Potsdam Analyzing the impact of herbicide exposure on ecosystem services provided by plants is gaining increasing importance in ecological risk assessment of pesticides. Stakeholders currently discuss the necessity to not only consider direct impacts of herbicides on plant individuals and populations but also effects emerging from inter- and intraspecific interactions, i.e. community-level endpoints such as diversity, structure and composition. We linked a spatiotemporally explicit drift exposure model (Xplicit) to an individual-based grassland community model (IBC-grass). Thereby we analyzed impacts of herbicide drift on structure and composition of non-target plant communities. Based on an extensive literature review of herbicidal field boundaries in Europe, a representative field-edge community of potential target plant species was generated. A spatiotemporal species drift model was set up on an IBC-grass trait-based approach. Variable herbicide exposure and its impact on individual-level was calculated with Xplicit and species effect distributions were generated. These individual-level effects were transferred to individuals of plants, seedlings and seeds within IBC-grass in order to simulate community effects of realistic herbicide drift events. Short-term effects of herbicide exposure on the structure and composition of the plant communities were closely linked to distance to field edge. On patches immediately at the field edge, the simulated plant functional type community showed a long-term change in structure and composition. A decrease in evenness indicates that abundance is more unevenly distributed among the PPFs. In contrast, with some meters distance only minor and transient effects could be detected. The mechanistic modelling approach indicates that the special and common and abundance within field boundaries can be impacted by agricultural practices such as herbicide applications. Modelling approaches such as IBC-grass can be useful tools to evaluate the importance of assessing community-level endpoints in real-world context in ecological risk characterizations.

TUPC33 Ecosystem services for aquatic macrophytes: linking ecology to risk assessment of chemicals G.H. Arts, Alterra Wageningen University and Research Centre / Environmental Risk Assessment; J. Wolters, University of Antwerp / Department of Biology Development of principles and methods based on the ecosystem services approach has been accepted in pesticide risk assessment recently. Ecosystem services research helps the benefits people obtain from ecosystem structures and processes. However, up to now, this ecosystems services approach has been applied more qualitatively than quantitatively. Risk assessment could benefit from a more quantitative approach i.e. the quantification of important ecosystem services. Here we focus on aquatic macrophytes as an example for service provision and research was performed in order to collate quantitative information related to ecosystem services provided by three different types of aquatic macrophyte vegetation: seagrass beds as a representative of submerged macrophyte vegetation; duckweed vegetation as a representative of floating macrophyte layers; reed as a representative of emergent macrophyte stands. Quantitative information was collated and categorized into provisioning, regulating, cultural and supporting services. These ecosystem services will be presented and used as an example how to link this information to the risk assessment of chemicals. Aquatic macrophytes deliver a number of ecosystem services that make them important service providing units (SPUs) in aquatic ecosystems. Provisioning services were common use and economically favourable in former times (e.g. mattress stuffing, thatching) and are becoming more popular recently (proteins). A number of regulating services include carbon fixation and storage, primary production and nutrient retention. In order link ecosystem services to risk assessment quantitatively, macrophyte assessment endpoints need to be linked to SPUs. Biomass seems to be a promising endpoint, as it is included as one of the assessment endpoints in the protection goals, can be linked to the ecosystem services provided by aquatic macrophytes and is an important output of macrophyte models assessing effects of chemicals. This approach needs further elaboration and quantification.

TUPC34 Further developments in the risk assessment of pesticides to non-target terrestrial plants M. Arena, EFSA / European Food Safety Authority / Pesticides; D. Auteri, Auteri / Pesticides Unit; A. Ippolito, EFSA / European Food Safety Authority / Institute for Environment and Sustainability IES; R. Luttik, R. Sharp, EFSA / European Food Safety Authority / Pesticides Unit; F. Streisål, EFSA / Pesticides Unit; C. Szentes, Pesticides Unit The EFSA Panel on Plant Protection Products and their Residues (PRPs) issued in 2014 an opinion on the state of the science for the risk assessment for non-target terrestrial plants (NTTPs) as a precursor to developing a new guidance document. The general protection goal for NTTPs is the maintenance of biodiversity in the agricultural area. Specific protection goals based on ecosystem services were identified for both the off-field and in-field NTTPs including endangered plant species. Non target terrestrial plants (NTTP) Assessment; M. Dollinger, Bayer CropScience; E. Kohlschmid, Agroscope / Alterra Wageningen University and Research Centre / Environmental Risk Modelling the impact of herbicide exposure on the structure and composition of non-target terrestrial plant communities T. Schad, EnSA; K. Körner, University of Potsdam; A. Solga, Ecology; T.G. Preuss, Bayer CropScience / Environmental Modelling; F. Jeltsch, University of Potsdam; T. Schad, EnSA; K. Körner, University of Potsdam; A. Solga, Ecology; T.G. Preuss, Bayer CropScience / Environmental Modelling; F. Jeltsch, University of Potsdam Analyzing the impact of herbicide exposure on ecosystem services provided by plants is gaining increasing importance in ecological risk assessment of pesticides. Stakeholders currently discuss the necessity to not only consider direct impacts of herbicides on plant individuals and populations but also effects emerging from inter- and intraspecific interactions, i.e. community-level endpoints such as diversity, structure and composition. We linked a spatiotemporally explicit drift exposure model (Xplicit) to an individual-based grassland community model (IBC-grass). Thereby we analyzed impacts of herbicide drift on structure and composition of non-target plant communities. Based on an extensive literature review of herbicidal field boundaries in Europe, a representative field-edge community of potential target plant species was generated. A spatiotemporal species drift model was set up on an IBC-grass trait-based approach. Variable herbicide exposure and its impact on individual-level was calculated with Xplicit and species effect distributions were generated. These individual-level effects were transferred to individuals of plants, seedlings and seeds within IBC-grass in order to simulate community effects of realistic herbicide drift events. Short-term effects of herbicide exposure on the structure and composition of the plant communities were closely linked to distance to field edge. On patches immediately at the field edge, the simulated plant functional type community showed a long-term change in structure and composition. A decrease in evenness indicates that abundance is more unevenly distributed among the PPFs. In contrast, with some meters distance only minor and transient effects could be detected. The mechanistic modelling approach indicates that the special and common and abundance within field boundaries can be impacted by agricultural practices such as herbicide applications. Modelling approaches such as IBC-grass can be useful tools to evaluate the importance of assessing community-level endpoints in real-world context in ecological risk characterizations.

TUPC35 An ecosystem services approach to pesticide risk assessment and risk management of non-target terrestrial plants: recommendations from a SETAC Europe workshop L. Malby, The University of Sheffield / Dpt of Animal Plants Sciences; G.H. Arts, Alterra Wageningen University and Research Centre / Environmental Risk Assessment; N. Dollinger, Bayer CropScience; E. Kohlschmid, Agroscope / Alterra Wageningen Institute for Plant Production Sciences IPS; H. Ochoa-Acuna, DuPont Crop Protection / Veterinary Pathobiology; V. Poulsen, ANSES / French Agency for Food Environmental and Occupational Health Safety The registration of Plant Protection Products (PPP) in the EU is under Regulation 1107/2009, which recommends a tiered approach to assessing the risk to non-target terrestrial plants (NTTPs). However, little information is provided on how to perform and implement higher tier studies or how to use them to refine the risk assessment. Therefore a stakeholder workshop was organized to consolidate current knowledge and expertise to aid the further development of testing and assessment procedures for NTTPs. The agreed recommendations of the workshop relate to the three main themes, i.e. specific protection goals, risk assessment and mitigation. The participants of the workshop adopted the European Food Safety Authority (EFSA) approach of using an ecosystem services framework for identifying specific protection goals. First, delivery and protection of ecosystem services were discussed for in-crop, in-field and off-crop, and off-field areas. Second, lower and higher tier risk assessment methods, including modelling approaches were evaluated. Third, options for raising the conversion of drift and run-off were discussed and evaluated. NTTPs provide a wide range of provisioning, regulating, cultural and supporting ecosystem services and may occur in-crop, off-crop/in-field and off-field. The workshop participants agreed that the type and relative importance of ecosystem services provided by NTTPs differ between different areas both in field and off field. A number of higher-tier options were identified and the benefits from these options addressed. A number of concerns were raised around these options and actions taken in order to reduce uncertainty. For the initial tiers, concern was especially raised around uncertainty related to test species (are standard test species protective for wild species?) and endpoints (are current regulatory endpoints protective of reproductive endpoints ?). At the level of field- or other multispecies-studies, participants concluded that these studies pose a challenge due to limited experience with this type of study and the absence of guidelines (what to measure and how ?). Related to exposure, the main question was what is the relative importance of different exposure pathways to non-target terrestrial plants ? These questions were translated into specific actions including collating and reviewing data and literature. The workshop report is foreseen for the

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Water acidification, temperature increases and changes in seawater salinity are common biochemical consequences linked to UVR, but not yet to DOC, is DNA damage from UV radiation (UVR),...{

Multistress in aquatic environments: the big picture (PC)

WEPC01

Effects of environmental stress on the genomic integrity of Daphnia magna (Straus, 1820)

K. Wall, Department of Biosciences; T. Andersen, University of Oslo; D.O. Hovestad, University of Oslo / Department of Biosciences; K. Hylland, University of Oslo / Biosciences

Introduction. Climate change is predicted to cause “browning”, i.e. an increase of dissolved organic carbon (DOC) in aquatic ecosystems. DOC offers protection from UV radiation (UVR), but may itself pose radical stress. One of the most common biochemical consequences linked to UVR, but not yet to DOC, is DNA damage. Using the limnetic cladoceran Daphnia magna, this damage is investigated by applying the comet assay, a single cell gel electrophoresis that can account for both single and double strand breaks. A more detailed feature linked to genomic damage is histone modifications such as γH2AX, which mediates DNA repair response. Our study investigates the influence of DOC and UVR on DNA damage and γH2AX levels in Daphnia. Materials and methods. Adult Daphnia magna were placed in 2 ml 90-well plates, and exposed to DOC (10 mg L\(^{-1}\)), UVR (λ = 390 nm), or a mixture of both. The comet assay was performed using a modified, high-throughput protocol. Investigation of histone modifications was carried out using standard western blot and semi-quantitative ELISA techniques. Results and discussion. DNA damage in control groups remained low, while UVR resulted in the highest increase of damage. This effect was buffered in a co-exposure to both DOC and UVR, while DOC itself also led to increased damage. Statistical differences were found between all exposure scenarios. We also showed the effects of UVR and DOC on histone modifications. Using antibodies specific towards human γH2AX, we demonstrated the existence of this histone modification in Daphnia. We also investigated the influence of different climate change scenarios on γH2AX. The results of these scenarios showed the trends for the comet assay and histone modification. Conclusions. Our study shows that exposure to DOC and UVR have detrimental effects on the genomic integrity of Daphnia. In a broader context, it is important to address how changes in the environment may potentially alter the effects of pollutants in aquatic ecosystems. Elevated levels of DOC imply an increased mobilisation and export of terrestrially derived heavy metals and organic pollutants. Our study demonstrates the complexity of stress responses, and by connecting established biomarkers with up-and-coming molecular methods, we work towards putting the way for future research by further bridging the gap between ecology and toxicology.

WEPC02

The effects of salinity, temperature and acidification on the regenerative capacity of the polychaete Dิepha neapolitana

A. Pires, Universidade de Aveiro / Biologia; E. Figueira, CESAM University of Aveiro; A. Moreia, CESAM Department of Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM; R. Freitas, Biology

Water acidification, temperature increases and changes in seawater salinity are expected to occur in the near future. Climate changes can induce risks for aquatic ecosystems, mainly on marine coastal areas, since they are inhabited by a large number of benthic species with ecologic and socio-economical importance. In this context, increasing efforts have been addressed to evaluate the effects of variations in temperature, salinity and pH on biological responses of marine organisms. Essentially due to their life-history characteristics, polychaetes have frequently been used to evaluate the effect of environmental disturbances in estuarine systems. The onuphoid polychaete Diapedia neapolitana represents a widespread spatial distribution, inhabiting intertidal and shallow subtidal habitats and frequently used as fish bait. In previous studies this species demonstrated to be a good bioindicator of metal contamination and organic matter enrichment. However, most of the studies were devoted to bioaccumulation patterns and biochemical alterations, and little is known on the effects of environmental stress on the regenerative capacity, due to environmental changes. Thus, the present study aims to assess the effects of temperature shifts, pH reduction and salinity changes in the tissue’s regenerative capacity of this polychaete species. The results obtained demonstrated that all the experimental conditions tested (pH, salinity and temperature) significantly affected the regenerative ability of the polychaete D. neapolitana. This study evidenced that D. neapolitana individuals exposed to lower pH exhibited significantly lower capacity to regenerate their body, while with the increase of temperature individuals showed a higher capacity to regenerate their tissues. Furthermore, the present work demonstrated that individuals exposed to salinities 28 and 35 did not present significant differences between them, while salinities 21 and 42 influenced negatively the regenerative capacity of D. neapolitana. At the end of the experiment, salinities 28 and 42 seemed to have a greater impact on the regenerative capacity of individuals than the other factors, since under this condition individuals took longer to completely regenerate. Overall, this study demonstrated that variations in abiotic factors can strongly affect D. neapolitana’s species performance. Keywords: Polychaetes, body regeneration, biomarker, climate change

WEPC03

Metal contamination of in situ caged Gammarus fossarum as an indicator to predict ecological disturbance: a national scale case-study

A. Cilibrifi, Irsste Lyon / UR MALY Laboratoire Ecotoxicologie; A. Chandessis, Irsste Lyon / UR MALY; M. Coqueret, Irsste Lyon / UR MALY; R. Recoura-Massagant, Irsste centre de Lyon Villeurbanne / UR MALY

Laboratoire Ecotoxicologie; Q. Chabanne, Irsste Lyon; A. Francois, Irsste centre de Lyon Villeurbanne / UR MALY Laboratoire Ecotoxicologie; a. chaumont, O. Geffard, Irsste / UR MALY Laboratoire Ecotoxicologie

Comprehension and prediction of ecological effects related to environmental disturbances calls for the development of a two-tier approach to: surveillance and validation of existing methods. As regards to metals, the prediction of ecological disturbance through the assessment of bioavailability is limited to the use of approaches not successfully validated in situ. Metal-related risk assessments should integrate results from field studies about the relationship between bioavailable concentrations and ecological response. In this aim, biota (which reflect bioaccumulation and bioavailability) should be used to estimate weight of metal contamination level, and 2/ the relationship between concentrations in biota and ecological response should be characterised. The validation of this approach at a wide scale could reveal the existence of a universal threshold for metal concentrations in a given biomonitor above which adverse ecological effects appear. Previous studies mainly rely on passive biomonitoring: the presence of the test organism is required in situ and the influence of confusing factors may be neglected. Active biomonitoring with transplanted organisms collected from the same population enables to overcome these drawbacks. Our work aimed at calibrating the active biomonitor Gammarus fossarum by relating the concentrations measured in in situ caged organisms to local ecological indicators. 129 sites were investigated in France, for which relevant ecological and chemical data were available. Controlled batches of gammarids were exposed during 7 days. Metals were analysed by ICP-MS (Cd, Pb, Ni, Co, Ag, As, Zn, Cr, Cu) or automated AAS (Hg). For most metals, bioaccumulation was significant with ratios between the lowest and the highest values related to the essential or non-essential character of the metals, confirming the relevance of this species tomonitor metal contamination in freshwater ecosystems. For all metals, results showed no relationship between bioaccumulation levels and global ecological disturbance. On the contrary, for Cd and Pb, a clear relationship was established between the caged organisms and the abundance of metal-sensitive invertebrates. Metal contamination in G. fossarum can be quantitatively related to metal-specific adverse responses in stream invertebrates. Dose-response curves, defined for each metal at the national scale, indicate that contamination measured in caged organisms could be useful to manage the risk of metallic contamination.

WEPC04

Bioaccumulation of organic pollutants in present-day and climate altered food webs

A.M. Berglund, Umea University / Department of Ecology and Environmental Science; M. Ripsam, Umea University / Department of Chemistry; C. Gallamois, Umea University / Chemistry; A. Andersson, Umea University / Ecology and Environmental Science; E. Griineide, D. Figueroa, P. Byström, Umea University / Department of Ecology and Environmental Science; P. Haglund, Department of

Literature data recording endpoints of crop and of wild species were collected, implemented in a database and merged with regulatory data provided by the Crop Life International NTP group, resulting in a dataset as comprehensive as possible. A total of 2484 species/a.s. test combinations were included in the database, some with just one, others with several endpoints (e.g. ER25, ER50, shoot height, biomass etc.), comprising 49 herbicides. Endpoints of crop and wild species were compared individually and differently depending on endpoint types. The majority of data was available for ER25 and ER50 endpoints, based on biomass measurements of vegetative vigour lab and field studies, hence the assessment focussed on these. The assessment was based either on the most sensitive species each, or on the average sensitivity of the group. Differences in sensitivity were expressed by means of a quotient, dividing the crop endpoint by the wild species endpoint. Quotients below “1” indicate cases where crop species were more sensitive than wild species (i.e. crop endpoints lower than wild species’ endpoints), quotients above “1” indicate that wild species were more sensitive than crop species. Average quotients were found generally to be close to 1, with deviations in both directions. The overall conclusion was that, based on ER25 and ER50 endpoints (biomass VV) and the available extensive data sets, no consistent differences in sensitivity between crop species and wild plant species were detected.
We examined the effects of a warming climate on the accumulation of organic pollutants (OPs) in the Baltic Sea. This region has a long history of pollution and is highly influenced by river run-off. With the proposed changes in climate (increased temperature and precipitation) a larger river discharge to the sea is expected, hence an increased inflow of OPs and dissolved organic matter (DOC) of terrestrial origin. Earlier studies have shown that increased temperature and DOC results in a shift at the base of the food web, favoring bacteria over phytoplankton. This creates a web with more trophic levels. Thus, in theory, top consumers in a bacteria-based food web could be subjected to pollutants at higher concentrations than in a phytoplankton dominated system, provided that the contaminants biomagnifies. In this mesocosm-study we exposed a marine community (from bacteria and phytoplankton to fish) to a cocktail of legacy and emerging OPs, using different climate scenarios (increased temperature and DOC). We found evidence for a bacteria favored food web with increasing DOC; but the bioaccumulation factor (BAF) of OPs were not higher in fish from DOC-treated mesocosms. If any, higher temperatures resulted in increased BAFs in fish but at higher temperatures DOC generally reduced the bioaccumulation. Thus, increased temperature may be an important factor for bioaccumulation of OPs in fish and the effect of DOC is complex and needs further investigations.

**WEPC05**

Linking multiple stressors to microbial community structure for water quality assessment

Freshwater quality assessment in Europe is established under the Water Frame Directive (WFD) based on measurements of chemical pollutants in sediments and water and biota (chemical status) and on the ecological indicators such as nutrients, temperature, species compositions etc. (ecological status). However in the current strategy a link between chemical and ecological status is missing. No biological indicators are foreseen to integrate the different anthropogenic pressures including chemical mixtures, nutrients, temperature changes in order to provide a global scenario on the ecosystem and therefore on the water quality. In this context, MicroCokit FP7-PEOPLE-2012-IAPP project is focused on the identification of microbial community based indicators for monitoring and evaluating the complexity of multiple stressors (e.g. chemical pollutants, temperature, nutrients etc.) relevant to guide preventive and remediation actions by the water authorities. For this purpose the river Tiber has been chosen as a pilot case study and sampling sites were selected based on different anthropogenic pressures which they are exposed to. Water samples are being analyzed for both microbiological (FISH analysis and Metagenomic) and chemical analysis (organic and inorganic compounds, including emerging pollutants). Within the 4-years project, two campaigns are contemplated per year (Autumn and Spring). We report preliminary results for the chemical compositions (inorganic elements, PAHs, organochlorines triazine, chloroacetamide pesticides, perfluorinated compounds, Pharmaceuticals, etc.) and epifluorescence direct microscopy analyses (total microbial abundance and phylogenetic community characterization by FISH) regarding the first two campaigns. The preliminary results suggest that the microbial community structure reflects both natural environmental variations (river course and sesonality) and anthropogenic pressures. The same water samples are currently being processed for metagenomic analysis in order to obtain a broader description of the microbial community compared to the overall chemical results.

**WEPC06**

Airborne pollution-driven basal feminization in natural lake trout populations from European mountain lakes
S. Jarque, Masaryk University / Faculty of Science RECETOX; E. Gallego, IDAEA-CSIk / Department of Environmental Chemistry; J. Grimalt, IDAEA-CSIC; B. Pina, IDAEA-CSIC / Environmental Chemistry

Gene expression levels of vitellogenin (Vtg) and Zona radiata protein 1 (Zrp) in liver were measured in brown trout (62 males and 81 females) from different lakes and brooks in four distant European mountain areas in Norway, Tyrol (Austria), Tatra Mountains (Slovakia/Poland) and the Pyrenees (Spain). As expected, expression of both genes was several orders of magnitude higher in mature females than in males, whereas females in the early stages of egg-formation showed levels of Vtg and Zrp expression close to male levels. Relatively high Zrp expression in males correlated with the presence of OCs in fish muscle, in agreement with previous reports that linked these compounds with estrogentic activity in fish muscle. Analysis of estrogen receptor expression in males corroborated the induction of estrogen-responsive genes in highly polluted male trout. Our data indicate an incipient feminisation of high mountain male trout, and constitute the first report of endocrine disruption associated exclusively to airborne pollution. The relatively mild nature of the observed feminisation suggests that it may not constitute a direct treat to the fertility of the impacted animals, but this situation may be clearly degraded if a massive redistribution of pollutants currently stored in the sediments should occur.

**WEPC07**

Workshop introduction, aims and objectives
S. Marshall, Unilever / SEAC

**WEPC08**

Aquatic exposures to chemicals in urban environments
S.D. Dyeg, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization

**WEPC09**

Use of prospective and retrospective risk assessment methods that simplify chemical mixtures associated with domestic discharge
M. Galav-Burgos, ECETOC

**WEPC10**

Assessment framework for the effects of chemical mixtures in the aquatic environment
S. Villar, University of Almeria; S. Martinez-Plenas, University of Almeria; A. Aguera, University of Almeria / Chemistry and Physics; P. Fernandez, IDAEACSIC / Environmental Chemistry; S. Malato, Plataforma Solar de Almeria-CIEMAT

**WEPC11**

Integrated prospective and retrospective exposure and risk assessment of chemicals from urban runoff, municipal waste water and agriculture
N. Aust, Federal Environment Agency (UBA) / Biocides

**WEPC13**

Determination of contaminants of emerging concern in soil substrate and plant material by QuEChERS extraction followed by liquid chromatography quadrupole linear ion trap tandem mass spectrometry
A. Martinez-Priem, University of Almeria; A. Aguera, University of Almeria / Chemistry and Physics; P. Fernandez, IDAEACSIC / Environmental Chemistry; S. Malato, Plataforma Solar de Almeria-CIEMAT

Presence of unregulated and not assessed contaminants of emerging concern (CECs) in wastewater effluents represents a significant challenge to wastewater reclamation. Problems associated to the repeated release of treated wastewater in the environment for reuse applications, such as irrigation practices, are still scarcely investigated. Therefore, the accumulation of contaminants in soils after irrigation and the evaluation of the uptake by crop plants are new insights that demand research in order to evaluate the possible risks for environment and human health. Consequently, comprehensive and high-throughput analytical methods have to be developed and validated to provide a comprehensive evaluation of these micromixtures in soils and crops. With this regard, the main difficulties associated to CECs analysis are the low concentration levels at which they are present in the complexity of the matrices which present great challenges due to high contents of pigments, and fatty or waxy materials, which may induce severe matrix interferences. Therefore, sample preparation methods able to eliminate potential interferences while permit to improve isolation and extraction of target compounds are required. In addition to conventional liquid extraction, other instrumental methods such as pressurized liquid extraction or microwave-assisted extraction have been applied to solid samples, but they usually require an additional clean-up step after extraction. QuEChERS extraction method has been adapted and optimized for the extraction of a set of CECs in both soil substrate and plant material (lettuce). Optimization of the dSPE clean-up step has been performed by using various phases (PSA, C18, ZSeph+). Efficiency was evaluated based on the recovery of the target analytes and matrix effects. The analysis was performed by liquid chromatography quadrupole-linear ion trap tandem mass spectrometry (LC-QqQ-LIT-MS). The analytical method has been validated with good results in...
Impact of urban wastes on prevalence of human opportunistic bacterial populations and antibiotic resistance genes in agricultural soils from Burkina Faso

B. Youenou, UMR CNRS / Laboratory of Microbial Ecology Université Lyon; S. Bouda, UFR SVT Université de Ouagadougou; E. Hien, UFR SVT Université de Ouagadougou / IIRD UMR EcoSols; E. Brothier, UMR CNRS / Laboratory of Microbial Ecology Université Lyon; S. Favre-Bonté, UMR CNRS 5557 Microbial Ecology Université Lyon; S. Nazari, UMR CNRS 5557 / Laboratory of Microbial Ecology Université Lyon

Spreading of municipal waste materials is a common practice among farmers of developing countries in sub-Saharan African areas and especially for urban and peri-urban agriculture. Few data are available on their impact on the prevalence of pathogens or antibiotic resistance genes (ARG) in soils under different pedo-climatic contexts than those in northern countries. This study examined the long-term effects of landfill disposal of untreated raw urban wastes on the prevalence of various human opportunistic pathogens (HOP) and ARG in soils from Burkina Faso in regards to the impact on physico-chemical properties. Soils were sampled in amended and unamended fields planted with sorgho at three sites in the periphery of Ouagadougou during campaigns conducted in 2008, 2011 and 2013. At the three sites amendment led to an increase in organic matter, pH, CEC, and metal content as well as a significant increase in total heterotrophic bacteria. No coliforms, Enterococci, Strepptococci and Staphylococci related indicators were detected. Regarding HOP P. aeruginosa was sporadically detected in the amended field at one site in 2011. At one site Bcc was detected in the amended fields in 2008 and 2011 and species belonging to B. cenocepacia, B. multivorans, B. dolousa, and B. latens were identified. S. maltophilia was detected at the 3 sites from the three campaigns, and at a higher level in amended fields. Antibiotic resistance tests showed that P. aeruginosa isolates and Bcc had a wild type phenotype. Regarding S. maltophilia multi-drug resistance was observed with resistance to 7 to 13 antibiotics comparable to the phenotypes of clinical isolates of reference strains. In conclusion, this study demonstrated that bioavailability of ARG from treated urban waste systems sulfonamides and quinolones resistance using a QPCR approach. qnrA, qnrB qnrS, blqAAG, and blqBAG were never detected neither in the amendments, nor in the amended and the unamended fields. On the opposite sul1, sul2, sul3, blqOSA, blqTAG, and blqBAG genes were detected at variable frequency and levels (10^2 to more than 10^7 copies / g soil dry weight) in amended fields and amendments at the 3 sites from all campaigns. These genes were rarely detected in the unamended soils. This study evidenced that amendment might impact directly the dissemination of pathogens and ARG-harboring populations by introducing them by exogenous sources but might indirectly alter their prevalence and properties by modifying environmental conditions.

WEPC17
Impact of meropenem and zinc levels on resistance development in biofilms in rotating tubular reactors

C.L. Hands. Civil Engineering and Geosciences; J.R. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; D.W. Graham, Newcastle University / Civil Engineering and Geosciences

Human morbidity and mortality has been substantially reduced since antibiotics were introduced to human therapy almost 60 years ago. However, the efficacy of antibiotics and their effect on public health is now being challenged by the emergence of antibiotic resistant bacteria. Many reports have indicated that elevated metal exposures can promote the development and proliferation of antibiotic resistance (AR). This experimental evidence from treatment systems and sludge/soil data show that AR was higher when zinc (Zn) levels were also high. In this study we measured and assessed changes in AR in microbial biofilm communities and effluents, by using an apparatus containing six aerobic rotating tubular reactors that mimic trickling filter beds, to treat domestic sewage with different levels of Zn and meropenem. Mixed domestic primary settled sewage and synthetic sewage was pumped through the reactors, to allow surface biofilms to develop and reach optimal thickness for 21 days, after which the following concentrations were added to each respective tubular reactor; no amendment (control), 2 mL/L meropenem (Reactor 2), 2 mg/L/L Zn (Reactor 3), 2 mg/L meropenem and 2 mg/L Zn (Reactor 4), 2 mg/L meropenem and 20 mg/L Zn (Reactor 5), 2 mg/L meropenem and 100 mg/L Zn (Reactor 6), and 2 mg/L meropenem and 2 mg/L Zn and 20 mg/L Zn (Reactor 5). Samples for microbiological, metal and molecular analysis were collected once a week from a common influent tank, six effluent flasks and from the biofilm at the top (end biofilm) and bottom end (lower biofilm) of each reactor and twice a week for chemical analysis. Microbiological analysis used R2A agar plates, amended with meropenem and Zn appropriately and molecular analysis used qPCR with appropriate probes and primers. The study showed that elevated Zn increased the prevalence of meropenem resistant bacteria in the biofilms, with an increase in total bacterial prevalence and dosage with 2 mg/L meropenem and Zn up to 100 mg/L. An average of combined meropenem and Zn resistance of 51% was seen when 100 mg/L of Zn was added, while 24% was seen when 20 mg/L of Zn was added, showing that increasing Zn levels result in increased meropenem resistance. The presence of meropenem in these reactors also increased resistance; however it is suspected the main reason for this increase was due to higher concentration of Zn dosed to the reactors. Moreover the presence of Zn alone, caused combined resistance as high as 5%. Elevated Zn levels in domestic sewage clearly increases meropenem resistance levels in treated effluents.
WEPC25
Aminomethylphosphonic acid (AMPA) chronic risk assessment for Daphnia and fathead minnow
G. von Mérey, Monsanto Europe S.A. / Regulatory; S.L. Levine, Monsanto Company / REgulatory Sciences; P. Manson, Cheminova AS European Regulatory Office / Global Regulatory Science; P. Sutton, Syngenta; H.O. Krueger, Wildlife International, Ltd. / Aquatic Toxicology; T. Minderhout, Evans Analytical Group Office / Global Regulatory Science; P. Manson, Cheminova AS European Regulatory

WEPC26
Can pyrethroid resistant Hyalella azteca accumulate ecologically-relevant pyrethroid tissue concentrations?
M.J. Lydy, Southern Illinois University-Carbondale / Center for Fisheries Aquaculture and Aquatic Sciences; A.D. Harwood, Southern Illinois University / Department of Natural Sciences; S. Natle, Southern Illinois University Carbondale / Zoology; L. Muggleberg, Southern Illinois University Carbondale / Department of Zoology

WEPC27
Effects characterization for aquatic invertebrates exposed to imidacloprid-Part B: Higher tier approaches
D. Moore, Intrinsic Environmental, Inc.; L. Knopper; R.L. Breton, Intrinsic Environmental Sciences, Inc.; C. Greer, Intrinsic Environmental Sciences (US), Inc. / Biovector; M. Ward, USDA Agricultural Research Service; T. Hall, Bayer CropScience / Environmental Toxicology and Risk Assessment; L.M. Bowers, Bayer CropScience / Environmental Safety

Risk assessment, risk management and mitigation for pesticides: from regulation to public perception (PC)
S. Nutile, Southern Illinois University Carbondale / Center for Fisheries Aquaculture and Aquatic Sciences; S. Nutile, Southern Illinois University Carbondale / Zool

Acute and chronic SSDs were derived and an HC5 calculated based on the Gumbel model. NOECs were typically determined from nominal concentrations and effects measured from two to 26 weeks. Typically two to four applications were made to the cosom separated by 7, 14 or 21 day intervals. As such, the mesocosm-based TSD can be considered a chronic effects metric. Given that the TSD was based on taxon NOECs, no further safety factors are required to derive the mesocosm-based chronic benchmark for aquatic invertebrates exposed to imidacloprid. These data can also be further assessed using other lines of evidence (e.g., modified Plant Assemblage Toxicity Index suitable for invertebrates) to derived a weight-of-evidence effects metric. For imidacloprid, a large number of higher tier studies (i.e., Tier 3 of good quality are available and produce a level of realism not found in laboratory studies. Using the higher tier evidence, the mesocosm-based TSD and other lines of evidence produced aquatic effects metrics for imidacloprid that are more ecologically relevant and have lower uncertainty than would effects metrics derived using lower tier approaches.

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are aggregated on 1sqkm hexagon level. We provide this habitat index for 22,529 tiles in total. The poster illustrates the spatial distribution of these habitats and gives example calculations for application patterns used in agricultural practice. The tool is implemented in a web-based advisory and cooperation system for farmers, beekeepers and nature conservationists to exchange relevant information (e.g., location of apiaries, insecticide treatment) while respecting data privacy among the partners. The concept was developed within the project "geobeet" which designs and implements a web-based information platform for the protection and promotion of wild bees and honey bee. It provides free of charge technical services and information content in order to promote dialogue and information exchange between beekeeping and nature conservation and agriculture.

**WEPC29**

**Five point plan for a sustainable use of plant protection products**


In 2009 the European Commission implemented the so-called “sustainable use directive” (Directive 2009/128/EC) in order to establish a framework for Community action to achieve the sustainable use of pesticides. Article 4 (1) of the directive requires: “Member States shall adopt National Action Plans to set up their quantitative objectives, targets, measures and timetables to reduce risks and impacts of pesticide use on human health and the environment and to encourage the development and intro-duction of integrated pest management and of alternative approaches or techniques in order to reduce dependency on the use of pesticides.” In Germany, the National Action Plan for sustainable use of plant protection products (PPP) was approved in spring 2013 by the government following an extensive multi-stakeholder debate. The German Federal Environment Agency (UBA) is in charge of the environmental risk assessment of PPP under Regulation (EC) No 1107/2009 as well as actively involved in the development and implementation of the German Action Plan. In this context, UBA developed five key principles / policy recommend-ations for a sustainable plant protection practice: Minimize PPP use Identify, quantify and communicate risks Optimize risk management Compensate inevitable ecological effects Internalize external costs The presentation will (i) explain the reasoning and motivation underlying these principles and (ii) discuss the actual and/or potential consideration of these principles in the National Action Plan and under the authorization procedure, respectively.

**WEPC30**

**Can the synergism of azole fungicides and pyrethroid insecticides observed in D. magna be quantified by measuring cytochrome P450 activity inhibition in vitro and in vivo?**

M. Gottardi, N. Cedergreen, University of Copenhagen / Department of Plant and Environmental Sciences

Cocktail effects of chemical mixtures can to a large degree be predicted, as long as the chemicals do not interact with each other physically, chemically or biologically. However, if interactions take place, synergy can occur, which is defined as the mixture giving a larger biological effect than predicted by a reference model. A recent study has shown that for more than 95% of the pesticides synergies for which the mechanism is known, one of the chemicals either stimulates or inhibits the activity of enzymes involved in xenobiotic detoxification pathways. A group of these enzymes, Cytochrome P450, plays the major role in oxidation of drugs and other foreign compounds in a vast variety of species and its activation or inhibition seems to play a key role for the mode of action of a range of synergizing chemicals. The question is whether the degree of synergy can be predicted based on the knowledge of how synergistic chemicals affect the activity of these key enzymes. The P450 inhibition potential of several azole fungicides such as prochloraz, propiconazole, tebuconazole and epoxiconazole and the known P450 inhibitor piperonyl butoxide, are investigated with fluorescence spectroscopy toward cytochrome P450 (ECOD) activity of commercially available rat liver microsomes in vitro and of D. magna in vivo. The experimentally found inhibition potentials, expressed as the concentration giving a 50 % decrease in enzymatic activity relative to the control (IC50) are compared with the synergism observed in a previous study (expressed as fold increase in toxicity). The ability of the inhibition potentials to predict observed synergism is then evaluated and quantified.
Chemical signalling.

Dioxins.

Cytotoxicity.

Decision analysis.

Cytotoxicity.

Degradation.

Depuration.

Desorption.

Development.

Ecotoxicology.

Endocrine disruption.
null
Urban.

161,179,31,32,35,45,46,47,48,62,64,73,96,97,M O196,M0201,M0216,M0228,M0230,M0231, M0242,M0243,M0244,M0245,M0246,M0248, M0250,M0251,M0272,M0273,M0365,MO 393,TH009,TH067,TU056,TU217,TU220,TU28, TU290,TU306,WE105,WE106,WE198,WE2 00,WE239,WE249,WE257,WE258,WE338,WE 350,WE417,WE418,WE419

Waste water.


Water quality.

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