Soaring to New Heights
Aiming for excellence in veterinary pharmacology
Program Committee

The AAVPT would like to thank the program committee below for their time, effort and dedication to the planning and execution of the 19th Biennial Symposium on Veterinary Pharmacology 2015: Soaring g to New Heights

Ronette Gehring, chair
    Anthony Lucas
    Sanja Modric
    Ralph Claxton
    Carol A. Davis
    Lesley Rausch-Derra
    Chantal Lainesse
    Maya Scott-Garrard
    Michele Sharkey
    Ben Moses
    Katherine Palmatier
    Luke Wittenberg

We hope that you will find the program informative and useful. We also hope that you will be interested in joining and/or being a more active member of the Academy. Enjoy the program!

For more information about the Academy, visit us at: http://www.aavpt.org/

AAVPT Past President
Sanja Modric, DVM, PhD
Sanja.Modric@fda.hhs.gov

AAVPT President
Anthony Lucas, BVMS, PhD
Anthony.lucas@putneyvet.com

AAVPT President-elect
Ronette Gehring, BVSc, MMedVet, Dip ACVCP
rgehring@ksu.edu

Secretary
Maya Scott-Garrard DVM, PhD
aavptsec@gmail.com

Treasurer
Ralph Claxton, DVM, MS
aavpttreas@gmail.com
BRONZE SPONSOR

KINGFISHER INTERNATIONAL

BAYER

invicro imaging services and software

AAVPT appreciates all the sponsors for their support!
The AAVPT is a professional organization that represents some of the most knowledgeable experts in veterinary pharmacology. Its mission is to promote the science of veterinary pharmacology and therapeutics. The AAVPT and its European counterpart, the EAVPT, are leading organizations where the top pharmaceutical scientists, pharmacologists and veterinarians in academia, government and industry can gather to explore the challenges facing the development and use of pharmaceuticals intended for use in veterinary species.

The objectives of the Academy are:

(a) To support and promote education and research in comparative pharmacology, clinical veterinary pharmacology and other aspects of pharmacology of interest to the veterinary profession;

(b) To sponsor a periodical Journal of the Academy that will publish reviews, summaries and original treatises on all aspects of veterinary pharmacology and therapeutics. The Journal of Veterinary Pharmacology and Therapeutics, hereinafter referred to as "the Journal", shall be an official instrument of the Academy;

(c) To sponsor and conduct workshops, symposia or other scientific and educational meetings pertaining to veterinary pharmacology and therapeutics;

(d) To enhance the exchange of educational materials and ideas among veterinary pharmacologists; and

(e) To organize committees of experts to research and make recommendations to the profession on current problems in veterinary therapeutics.
**BE A PART OF THE LEADERSHIP THAT TAKES VETERINARY PHARMACOLOGY INTO THE FUTURE**

The AAVPT offers its membership:

- State-of-the-art scientific programming.
- Development of position papers on hot issues (e.g., *The Evaluation of Scientific Manuscripts that Involve the Administration of Compounded Drugs to Animals; Post-Exposure Management and Treatment of Anthrax in Dogs*).
- Co-sponsorship of one of the première veterinary research journals, the Journal of Veterinary Pharmacology and Therapeutics.
- The only network consisting of pharmaceutical scientists and pharmacologists with Ph.D. and/or DVM background within the US.
- Very low membership fees, courtesy of utilizing a purely volunteer system for our activities. Associate Fellow dues $35, Fellow dues $45
- Access to the AAVPT Listserve for posting questions and for announcement of career opportunities.
- Opportunities to participate in collaborative events with other outstanding scientific organizations such as the American Association of Pharmaceutical Scientists (AAPS), the Controlled Release Society (CRS), The American College of Veterinary Internal Medicine (ACVIM), the American College of Veterinary Clinical Pharmacology (ACVCP), and the American Society of Pharmacology and Experimental Therapeutics (ASPET).

**Interested in joining AAVPT?** Membership forms are available at: [https://aavpt.site-ym.com/general/register_member_type.asp?](https://aavpt.site-ym.com/general/register_member_type.asp?)

Or Contact: Dr. Jonathan Hare  
Chair, AAVPT Membership and Bylaws Committee  
jhare@kingfisherint.com
The "Lloyd E. Davis Award" is presented for significant contributions over an entire career to the advancement and extension of knowledge in the fields of veterinary or comparative pharmacology. The AAVPT is honored to present the 2015 Lloyd E. Davis Award to Dr. Marilyn Martinez.

Marilyn N. Martinez, Ph.D., is a Senior Biomedical Research Scientist at the Food and Drug Administration-Center for Veterinary Medicine, where she has dedicated the last 30 years to the advancement of pharmacology. Dr. Martinez earned her B.S. and M.S. in Zoology/Mammalian Physiology from the University of Maryland. She earned her Ph.D. with high honors from the Department of Physiology and Biophysics at Georgetown University School of Medicine in 1983.

Dr. Martinez is a Fellow of the American Academy of Veterinary Pharmacology and Therapeutics and a Fellow of the American Association of Pharmaceutical Scientists. Additionally, Dr. Martinez has served as an expert, or chairperson, and is an active member of numerous professional organizations including the Controlled Release Society, Clinical Laboratory Standards Institute, the US Pharmacopeia, and the Veterinary International Conference on Harmonization.

Dr. Martinez has extensively published with over 65 peer-reviewed manuscripts and 15 book chapters to her credit. She has been invited to present at over 70 national and international conferences. Dr. Martinez currently serves on the editorial board of the Journal of Veterinary Pharmacology and Therapeutics, is an associate editor for the American Association of Pharmaceutical Scientists Journal, and serves as an Ad hoc reviewer for numerous peer-reviewed journals.

Dr. Martinez continues to advance veterinary and human pharmacology through countless research endeavors, teaching and student mentoring. She is the recipient of numerous FDA Awards, the 2013 AAPS Regulatory Sciences Recognition Award, and the 2011 AAVPT Service Award.
The Teaching Award is presented to recognize significant teaching activities in the fields of veterinary or comparative pharmacology, or therapeutics. AAVPT is proud to present this award to Dr. Sidonie Lavergne. Dr. Lavergne is an Assistant Professor of Pharmacology in the Department of Veterinary Biosciences at the University of Illinois. She is known for her passion, intensity of her instruction, and is a wonderful role model for students. Dr. Sidonie Lavergne has trained over 10 veterinary students, 15 undergraduate students, and 10 graduate students and/or residents in her career. For these efforts, in 2011, she was awarded the College's highest teaching award, the 2011 Dr. Gordon and Mrs. Helen Kruger Teaching Excellence Award.
AAVPT Biennial Symposium Agenda

Sunday May 17, 2015

1:00 – 4:00 PM  Arrival and Symposium check-in
4:00 – 4:30 PM  Careers in veterinary pharmacology
4:30 – 5:00 PM  Panel discussion
5:00 – 6:00 PM  Student Social Event

Monday May 18, 2015

7:30 – 8:30 AM  ACVCP Business Meeting
8:30 – 9:00 AM  BREAK

Session I – Welcome and Keynote Address

9:00 – 10:00 AM  Welcome
Dr. Anthony Lucas, AAVPT President

Keynote Address: VETERINARY ETHICS, ANIMAL WELFARE AND OUR UNDERSTANDING OF HUMAN-ANIMAL INTERACTIONS
Dr. Temple Grandin

10:00 – 10:30 AM  BREAK

Session II – Pain Management

10:30 – 11:00 AM  Pain management in food producing animals:
The challenges and current state of the art
Dr. Hans Coetzee

11:00 – 11:30 AM  Pain management in food producing animals:
An evidence-based approach
Dr. Annette O’Connor

11:30 – 12:30 PM  LUNCH

12:30 – 1:00 PM  Pain management in small animals
Dr. Marlis Rezende

1:00 – 1:30 PM  Approval of drugs for pain management in animals: an Industry perspective
Dr. Ralph Claxton
AAVPT Biennial Symposium Agenda

Monday May 18, 2015

Session II – Pain Management - continued

1:30 – 2:00 PM   Questions & Answers
2:00 – 2:30 PM   BREAK
2:30 – 3:00 PM   Update form Education Committee and student competencies
                  Dr. Virginia Fajt
3:00 – 3:15 PM   BREAK

Session III – VPRF Awardee presentations

3:15 – 3:30 PM   Effect of CYP inhibition on tramadol PK/PD in dogs
                  Dr. Butch KuKanich
                  Abstract: Published in Proceedings of 18th AAVPT Biennial Symposium

3:30 – 3:45 PM   Development of novel amikacin delivery method for granulomatous colitis in Boxers
                  Dr. Kenneth Simpson
                  Abstract: Not submitted

3:45 – 4:00 PM   PK/PD of apibaxin in cats
                  Dr. Jennifer Myers
                  Abstract: See Poster #19

4:00 – 4:15 PM   Exenatide extended release in cats
                  Dr. Glen Gilor
                  Abstract: See Poster #21

4:15 – 4:30 PM   Dose of terbinafine for Malassezia infections in dogs
                  Dr. Jacquelyn Gimmler
                  Abstract: See Poster #3

4:30 – 5:00 PM   Q&A/Overflow time

5:00 – 6:00 PM   BREAK
**AAVPT Biennial Symposium Agenda**

**Evening Social Event – Wine & Cheese reception**

6:00 – 8:00 PM  
Poster Session/ Wine & Cheese

**Tuesday May 19, 2015**

7:30 – 8:30 AM  
AAVPT Business Meeting
8:30 – 9:00 AM  
BREAK

**Session IV – Antimicrobial Therapy**

9:00 – 10:00 AM  
Antimicrobial therapy in food animals  
*Dr. Michael Apley*

10:00 – 11:30 AM  
Antimicrobial therapy in companion animals  
*Dr. Paul Morely*

11:30 – 12:00 PM  
Roundtable discussion

12:00 – 1:00 PM  
LUNCH

**Session V – Veterinary Oncology**

1:00 – 2:00 PM  
Pharmacodynamics in Veterinary Oncology: Present and Future  
Dr. Luke Wittenberg

2:00 – 2:30 PM  
BREAK

2:30 – 3:00 PM  
Outcomes in veterinary oncology  
*Dr. Doug Thamm*

3:00 – 3:30 PM  
Analyzing drug effects in cytotoxic versus targeted anticancer therapies: *in vitro* and *in vivo* translation  
*Dr. Dan Gustafson*

3:30 – 4:00 PM  
Roundtable discussion
AAVPT Biennial Symposium Agenda

Tuesday May 19, 2015 continued

4:00 – 4:45 PM     USP update
                  Drs. Dawn Boothe, Carol Davis and Gigi Davidson

4:45 – 5:00 PM     BREAK

Evening Social Event

5:00 -6:00 PM     VPRF Silent Auction

6:00 – 8:00 PM     Banquet and Awards Ceremony

Wednesday May 20, 2015

Session VI – Drug Compounding: The Good, the Bad and the Ugly

8:00 – 8:10 AM     Introduction
                    Dr. Chantal Lainesse

8:10 – 9:00 AM     Regulatory enforcements
                    Dr. Dawn Boothe

9:00 – 9:50 AM     USP standards
                    Dr. Gigi Davidson

9:50 – 10:40 AM    Pharmacist’s perspective
                    Dr. Richard Allen

10:40 –10:55AM    BREAK

10:55 – 11:45 AM   What to ask your compounding pharmacist
                    Dr. Jane Owens

11:45-12:35 PM    Clinician/clinical pharmacology perspective
                    Dr Patricia Dowling

12:35 – 1:00 PM    Roundtable discussion
MANUSCRIPTS
Challenges associated with providing analgesia in food animals

There are several challenges associated with providing effective analgesia in food animals in the United States. Firstly, there are currently no analgesic drugs specifically approved for the alleviation of pain in livestock. Therefore, use of any drug for pain relief constitutes extra-label drug use (ELDU). Under the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA), ELDU is permitted for relief of suffering in cattle provided specific conditions are met. These conditions include that (1) ELDU is allowed only by or under the supervision of a veterinarian, (2) ELDU is allowed only for FDA approved animal and human drugs; (3) ELDU is only permitted when the health of the animal is threatened and not for production purposes; (4) ELDU in feed is prohibited and (5) ELDU is not permitted if it results in a violative drug residue in food intended for human consumption. Therefore, use of an analgesic to alleviate pain associated with castration in calves in the United States would be required by law to comply with these regulations.

A second challenge to providing effective analgesia in cattle is that there is often a delay between the time of drug administration and the onset of analgesic activity. For example, local anesthetics require 2 - 5 minutes before a maximal effect is achieved. This may slow animal processing as producers must wait for local anesthesia to take effect. This delay may serve as a disincentive for them to provide routine preemptive analgesia. Furthermore, the requirement for large numbers of animals to be processed quickly may result in procedures being initiated before optimal analgesia is achieved. A third challenge is that the route or method of analgesic drug administration may require specialized training and expertise or may be hazardous to the operator. For example, the NSAID flunixin meglumine is only approved for IV administration in the United States. Therefore, administration requires the animal to be adequately restrained and the operator to be proficient in IV administration. Similar issues are encountered with epidural analgesic drug administration and administration of local anesthesia into the scrotum. The latter procedure is also considered especially hazardous by many livestock handlers. In addition, the majority of analgesic drugs that are available in the U.S. have a short elimination half-life necessitating frequent administration in order to be effective. This increases the stress on the individual animal and increases labor and drug costs.

In addition to the regulatory considerations discussed previously, certain drug classes such as the opioid and NMDA receptor antagonists are designated as Schedule 3 drugs and are subject to regulation by the U.S. Drug Enforcement Administration (DEA). Therefore administration of these compounds to provide pre-emptive analgesia is restricted to use by licensed veterinarians. Finally, the cost associated with providing preemptive analgesia contributes to the reluctance of producers to adopt these measures especially since there is no perceived economic benefit for doing so. It may also be difficult for producers and veterinarians to determine if analgesic...
compounds are effective because cattle may not show overt signs of pain and distress. Thus determining the need for analgesia and the dose, route, duration and frequency of drug administration in cattle can be especially challenging.

**Pain management options**

**Meloxicam**

Meloxicam is a NSAID of the oxicam class that is approved in the European Union for adjunctive therapy of acute respiratory disease; diarrhea and acute mastitis when administered at 0.5 mg/kg IV or SC. Meloxicam is considered to bind preferentially to cyclooxygenase-2 (COX-2) inhibiting prostaglandin synthesis although definitive evidence of COX-selectivity in calves is deficient in the published literature. Heinrich et al. (2009) demonstrated that 0.5 mg/kg meloxicam IM combined with a cornual nerve block reduced serum cortisol response for 6 hours in 6-12 wk old calves compared with calves receiving only local anesthesia prior to cauter dehorning. Furthermore, calves receiving meloxicam had lower heart rates and respiratory rates than placebo treated control calves over 24 hours post-dehorning. Stewart et al. (2009) found that meloxicam administered IV at 0.5 mg/kg mitigated the onset of pain responses associated with hot-iron dehorning in 33 ± 3 day old calves compared with administration of a cornual nerve block alone as measured by heart rate variability and eye temperature. These findings indicate that administration of meloxicam at 0.5 mg/kg IV or IM decreases physiological responses that may be linked to pain and distress associated with cauter dehorning in preweaning calves.

The purpose of this study was to investigate the pharmacokinetics and oral bioavailability of meloxicam in ruminant calves. Six Holstein calves (145 – 170 kg) received either meloxicam IV at 0.5 mg/kg or oral meloxicam at 1 mg/kg in a randomized cross-over design with a 10-day washout period. Plasma samples collected up to 96 hours post-administration were analyzed by LC-MS followed by noncompartmental pharmacokinetic analysis. A mean peak plasma concentration (Cmax) of 3.10 ug/mL (Range: 2.64 – 3.79 ug/mL) was recorded at 11.64 hours (Range: 10 – 12 hours) with a half-life (T ½ λz) of 27.54 hours (Range: 19.97 – 43.29 hours) after oral meloxicam administration. The bioavailability (F) of oral meloxicam corrected for dose was 1.00 (Range: 0.64 – 1.66). These findings indicate that oral meloxicam administration could be an effective and convenient means of providing long-lasting analgesia to ruminant calves.

In the United States, meloxicam administered to cattle by any route constitutes extra-label drug use (ELDU). Under the Animal Medicinal Drug Use Clarification Act (AMDUCA),ELDU is permitted for relief of suffering in cattle provided specific conditions are met. These conditions include that (1) ELDU is permitted only by or under the supervision of a veterinarian, (2) ELDU is allowed only for FDA approved animal and human drugs; (3) ELDU is only permitted when the health of the animal is threatened and not production purposes; (4) ELDU in feed is prohibited and (5) ELDU is not permitted if it results in a violative food residue. Therefore, use of oral meloxicam to alleviate suffering associated with dehorning and castration in calves in the United States would be required by law to comply with these regulations.

Extra-label use of a drug (ELDU) is administering a drug in a manner not specified on its label (eg, giving a higher dose, giving it more often than directed, giving it to a different type of animal than what it was approved for). ELDU must only be done by direction of a licensed...
veterinarian, and it is the responsibility of the prescribing veterinarian to ensure that meat or other food products from treated animals do not enter the food supply with unsafe residues. Practicing veterinarians do not always have the necessary information in order to determine safe withdrawal periods for ELDU. To help, both the United States and Canada have each developed services that provide recommendations to veterinarians when they must use drugs in an extralabel manner in order to practice good veterinary medicine. The services are cooperative but separate, as there are distinct differences in regulations between the two countries. In the USA, veterinarians can contact FARAD (Food Animal Residue Avoidance Databank) at (www.farad.org/).

Meloxicam is an approved injectable product (Metacam) for use in calves in Canada. Its label directions are quite specific: “As an aid in improving appetite and weight gains when administered at the onset of diarrhoea, in combination with oral rehydration therapy, in calves over one week of age. For relief of pain following de-budding of horn buds in calves less than 3 months of age.” Therefore use of the drug for the relief of pain from castration falls under the 2 step of the decision process and is supported as an effective and humane practice from our research studies. In the United States, Metacam is not available in an approved product for cattle, but there are injectable and oral formulations for dogs and cats. In the United States, the 2 step of the decision process for veterinarians is to choose to use an approved veterinary product in an extralabel manner OR an approved human drug. This is the basis of our work with using human-approved oral meloxicam tablets in calves to relieve the pain of castration.

Currently the only NSAID approved for use in cattle in the United States is flunixin meglumine. The plasma elimination half-life of flunixin is reported to be 3 – 8 hours therefore requiring once daily administration. Although this drug class is recognized as having analgesic properties, flunixin is only indicated for control of fever associated with respiratory disease or mastitis, and fever and inflammation associated with endotoxemia, rather than for control of pain. Studies demonstrating the analgesic effects of flunixin at the approved dose of 2.2 mg/kg are deficient in the published literature. Use of flunixin meglumine is further complicated by the requirement for intravenous administration which is more stressful on the animal and involves more skill and training on the part of the operator. Several reports have suggested that the IM administration of flunixin may result in significant myonecrosis and tissue residues. In the absence of data demonstrating that flunixin reduces signs of pain and distress associated with dehorning and castration in calves, it could be argued that use of oral meloxicam for this purpose can be justified under AMDUCA. Meloxicam (20 mg/ml) is approved for use in cattle in several European countries with a 15 day meat withdrawal time and a 5 day milk withdrawal time following administration of 0.5 mg/kg IM or SC. An oral meloxicam suspension (1.5 mg/mL) and injectable formulation (5 mg/mL) are approved in the United States for the control of pain and inflammation associated with osteoarthritis in dogs. Furthermore, an injectable formulation (5 mg/ml) is approved for the control of post-operative pain and inflammation in cats. Several generic tablet formulations containing meloxicam (7.5 and 15 mg) have recently been approved for relief of signs and symptoms of osteoarthritis in human medicine. The cost of administering IV meloxicam to calves in the present study was approximately US $58.00/100 kg bodyweight and the cost of administering oral meloxicam was US $0.30/100 kg bodyweight.

Gabapentin
Gabapentin is a γ-aminobutyric acid (GABA) analogue indicated for treatment of neuropathic pain. This study determined the pharmacokinetics of oral gabapentin alone or in combination with meloxicam in ruminant calves. Gabapentin capsules at 10 mg/kg PO or gabapentin powder (from capsules) and meloxicam tablets at 15 mg/kg and 0.5 mg/kg PO, respectively was administered to six beef calves. Plasma drug concentrations were determined over 48 h post-administration by liquid chromatography/mass spectrometry followed by non-compartmental pharmacokinetic analysis. The mean (±SD) Cmax, Tmax and elimination half-life (t½ λz) for gabapentin (10 mg/kg) alone was 2.97±0.40 µg/mL, 9.33±2.73 h and 11.02±3.68 h, respectively. The mean (±SD) Cmax, tmax and t½ λz for gabapentin (15 mg/kg) co-administered with meloxicam was 3.57±1.04 µg/mL, 7.33±1.63 h and 8.12±2.11 h, respectively. The mean (±SD) Cmax, Tmax and t½ λz for meloxicam was 2.11 ± 0.19 µg/mL, 11.67 ± 3.44 h and 20.47 ± 9.22 h, respectively. Plasma gabapentin concentrations >2 µg/mL were maintained for up to 15 h and meloxicam concentrations >0.2 µg/mL for up to 48 h. The pharmacokinetic profile of oral gabapentin and meloxicam supports clinical evaluation of these compounds for management of neuropathic pain in cattle.

A study was conducted to examine the pharmacokinetics and analgesic effect of oral meloxicam (MEL) administered alone or in combination with gabapentin (GABA) in an experimental bovine lameness model. Eighteen male British × Continental beef calves aged 4-6 months and weighing 297 - 392 kg were randomly assigned to receive either (1) 0.5 mg/kg lactose monohydrate placebo (PLBO) (n=6); (2) 0.5 mg/kg MEL (n=6); or (3) 0.5 mg/kg MEL combined with 15 mg/kg GABA (n=6) q24h for 4 days. The first treatment was administered 4 h after a chemical synovitis/arthritis was induced with injection of 15 mg amphotericin B into the left hind distal interphalangeal joint. Changes in activity were evaluated continuously with pedometers. Contact force, contact area, contact pressure, impulse and stride length were recorded once daily with a pressure mat and visual lameness scores were determined by a masked observer using a 5-point scale. Cortisol and drug concentrations were determined daily by immunoassay and HPLC-mass spectrometry respectively. Outcomes were compared statistically using a random effects-mixed model and ANCOVA. There was a positive association between lameness scores and serum cortisol concentrations (P = 0.02) and a negative association between lameness score and step count (P < 0.0001), total force (P = 0.001), force applied to the lateral claw (P = 0.02), contact pressure (P = 0.005) and impulse of the lateral claw (P = 0.01). Step count was greater in MEL calves compared with PLBO (P = 0.008) and MEL-GABA (P = 0.04) calves. Impulse was greater in the MEL-GABA calves compared with the PLBO calves (P = 0.03). There was an inverse relationship between plasma MEL concentrations and lameness score (P = 0.02) and a positive association between MEL concentrations and force applied to the lateral claw (P = 0.03), total contact pressure (P = 0.03) and impulse on the lateral claw (P = 0.02). There was a tendency towards a positive association between GABA concentrations, total impulse and impulse on the lateral claw (P = 0.08) and a negative associate between GABA concentrations and step count (P = 0.08). The results of this study suggest that MEL administered alone or in combination with GABA reduced the severity of lameness in calves following induction of lameness with amphotericin B. These findings have implications for developing analgesic protocols in lame calves that address both production and welfare concerns.
Transmammary delivery of meloxicam

To investigate a novel route of providing analgesia to processed piglets via transmammary drug transfer, meloxicam was orally administered to sows after farrowing. The objectives of the study were to demonstrate drug transfer from sows to piglets and to describe the analgesic effects in piglets through pain biomarkers and infrared thermography (IRT). Ten sows received either meloxicam (30 mg/kg) or an equivalent volume of whey protein (placebo) in their daily feedings, starting at four days after farrowing and continuing for three consecutive days. During this time frame, blood and milk samples were collected at 12-hour intervals. On day 5 after farrowing, three boars and three gilts from each litter were castrated or sham castrated, tail docked, and given an iron injection. Piglet blood samples were collected immediately before processing and at predetermined times over an 84-hour period. IRT images were captured at each piglet blood collection point. Piglet plasma was tested to confirm meloxicam concentrations using a validated high-performance liquid chromatography mass spectrometry (HPLC-MS) method. Meloxicam was detected in every meloxicam-treated piglet at each time point, and the mean (±standard error of the mean) meloxicam levels at castration were 568.9 ±105.8 ng/mL. We demonstrated that PGE₂ production is significantly inhibited ex-vivo in blood samples that were collected from piglets nursing on meloxicam-treated sows (p=0.0059). Arachidonic acid is cleaved by cyclooxygenase (COX) to generate PGE₂. The analgesic and anti-inflammatory effects of NSAIDs are associated with COX inhibition as determined by suppression of PGE₂ synthesis.
There was a time-by-treatment interaction for processed piglet serum cortisol (p=0.0009), with meloxicam-treated piglets demonstrating lower cortisol levels for 10 hours after castration than control piglets. The acute cortisol response is used to determine the extent and duration of castration-associated distress in pigs. IRT demonstrated significant changes in cranial temperature between meloxicam and placebo-treated piglets (p=0.015). IRT evaluates changes in surface temperature. Processing can release epinephrine, which causes changes in sympathetic tone. The adrenergic effects on cutaneous blood flow results in a decrease in cranial skin temperature after castration. These changes can be quantified with a thermography camera. In this study we demonstrated that piglets nursing on a meloxicam-treated sow (A) had higher cranial skin temperatures after processing than piglets nursing on a placebo-treated control sow (B), as illustrated in Fig.C.

**Acknowledgements**

Supported by the USDA- CSREES Animal Protection (Animal Well-being) NRI Grant (No. 2008-35204-19238).

**References available upon request**
APPRAOCHES TO EVALUATING PAIN MITIGATION IN PIGLETS

A.M. O’Connor
Iowa State University

Acknowledgment


The final versions of the studies discussed here are available open access @ Animal Health Research Reviews (1, 2)

Funding

This project including open access publication costs were funded by the National Pork Board (NPB) grant #12-186.

Introduction and motivating example

Reviewing the evidence and developing recommendations is not a new process in veterinary science. However, as stakeholders become increasingly involved in science, so has the call for transparent and comprehensive approaches to research synthesis and guideline development. Consequently new methods of research synthesis and guideline development with an emphasis on transparency are increasingly used in human health. The US Agency for Healthcare Research and Quality (AHRQ) (http://www.ahrq.gov/), the National Guidelines Clearing House (http://www.guideline.gov) and Patient-Centered Outcomes Research Institute (http://www.pcori.org/about-us) are all examples of the new focus on transparent guidelines. The advantages of transparent approaches to research synthesis and guideline development is a reduction in the wastage of resources by ensuring all research is considered in the development of guidelines and also identify over studied areas, or research gaps. Further, as stakeholders are able to see the basis for inclusion or exclusion of data, the outcomes from transparent processes are often more accepted and adopted.

In livestock production there are numerous practices where there is debate about the use of interventions. In swine production, piglets undergo castration, tail docking, teeth clipping, and identification with ear notching or ear tagging. These procedures are considered painful. Although available and increasingly warranted by the public in other countries, pain mitigation strategies during these procedures are not routinely provided to piglets in the United States (US). Further, although numerous studies have been conducted to assess how pharmaceutical products mitigate pain in this population, no systematic review had been conducted. Therefore, the purpose of this project was to summarize the literature, identify gaps in the literature for pain
mitigation in piglets and develop recommendations based on that evidence. Given the public concern about pain procedures in livestock production, it was felt that transparent approach to decision making was necessary, to ensure that all stakeholders (producers, consumers and others) where aware of the factors that where considered in the process of reaching a decision.

The overall project had two objectives. The first objective was to summarize the findings reported in studies that assessed pain mitigation strategies in piglets for routine i.e., castration, tail docking, teeth clipping, and ear notching. The second objective was to develop recommendations for the use of pain mitigation strategies in piglets for US based swine production systems while explicitly using the findings from the review including the effect size and potential for bias in the body of work available.

**Methods and materials**

The approach to the 1st objective was to conduct a systematic review to synthesize the existing primary scientific literature regarding the effectiveness of pain management interventions used for routine procedures on piglets. For the 2nd objective the GRADE approach to decision-making was employed. The GRADE approach emphasizes four aspects of decision-making – the risk of bias in the scientific evidence, the balance of benefits and harms, values and preferences and resources.

Recommendation development is a multi-step process Oxman, Schunemann (3) and includes a systematic review. For the systematic review we followed the approach described by the European Food Safety Authority (4, 5). The specific review question was, “In piglets that undergo castration, tail docking, teeth clipping and/or methods of identification that involve cutting of the ear tissue, such as ear tagging and ear notching, what is the effect of pain mitigation (e.g., general anesthesia, local anesthesia, NSAIDs), compared with no pain mitigation, on behavioral (e.g. postures, vocalizations) and non-behavioral (e.g. blood cortisol, norepinephrine, β-endorphin levels) indicators of procedural pain, assessed within sixty minutes of the procedure, and post-procedural pain, assessed between one and twenty four hours of performing the procedure?”.

For the development of guidelines The Grading of Recommendations Assessment, Development and Evaluation (GRADE) process. Examples of guidelines developed using this approach have been published by the World Health Organization1, the American College of Physicians2, the Agency for Healthcare Research and Quality3, and the US Centers for Disease Control and Prevention4. The GRADE approach is also extensively described in a series of publications, and revised periodically (6-22).

In O’Connor et al (2) the GRADE approach is summarized as followed: “The process acknowledges the importance of scientific evidence and the potential for biased scientific information. It articulates ethical and non-ethical values and preferences that motivate recommendations. A key concept in the development of recommendations is that scientific evidence is global, but decisions are local. Scientific evidence is considered to be global in that if

---

4 [http://www.cdc.gov/vaccines/recs/acip/GR Ide/recommendations.pdf](http://www.cdc.gov/vaccines/recs/acip/GR Ide/recommendations.pdf)
reviewers use the same approach for searching, extracting, and analyzing data during the review process, they typically arrive at the same conclusions. Decision-making is local in that it is informed by local challenges, values, and preferences, and by the limitations of a particular review process.”

A schematic of the entire review and guideline development process is presented in Figure 1. It can be seen that the process begins with identification of a question by a panel of experts, followed by a research synthesis process conducted by a review team (a series of systematic reviews for each critical outcome) and ends with the guideline development which again involves the panel of experts. The resulting recommendations are presented in summary of findings tables and the evidence summary for each outcome and an overall recommendation table that combines all the data across the outcomes. (8, 9, 11, 23, 24).

Results and discussion:

The results of the systematic review and the final recommendations are published as open access publications elsewhere and only presented briefly here(1, 2). Only 40 studies described mitigation of pain for castration. For the other procedures too few studies were available for synthesis. One major issue was the many outcomes used by many authors was not considered critical indicators of pain. In particular, cortisol was frequently reported but not considered to be a critical indicator of pain by the expert panel. This weakened the ability to make strong recommendations for products. Unfortunately, the extent of comprehensive reporting of the studies was also poor. Frequently omitted information included the magnitude of the effect size that compared treatment groups or measures of precision. This meant it was difficult to incorporate all the information from the studies into the review and decision making process. Poor reporting is a concern because it constitutes research wastage if results can not be included in the decision making process(25-27). The main recommendation main from the review was as followed “ The panel’s current recommendation is a weak recommendation for the use of NSAIDS for pain mitigation during castration of piglets 1 to 28 days of age.” (2). The quality of evidence for this recommendation was considered low, however it was considered that the benefits outweighed the harms. The major limitation was as follows “Currently, the absence of an FDA-registered product for pain mitigation is a major barrier that must be resolved. The primary impediment to regulatory approval for pain indications is the absence of validated methods for pain assessment in swine. Similarly, several of the products under consideration are considered prescription drugs. Such a designation makes their widespread use in production settings more difficult and expensive to manage. “(2).
References

5. EFSA. EFSA Journal. 2010; 8:1-90
PAIN MANAGEMENT IN SMALL ANIMALS

Marlis Rezende
Colorado State University
FDA APPROVAL OF PAIN DRUGS FOR ANIMALS: 
AN INDUSTRY PERSPECTIVE

R. F. Claxton

Development steps leading to FDA Center for Veterinary Medicine (CVM) new animal drug approval ideally start with a rational chemistry concept and end in achieving market authorization based on legally defensible (i.e., high evidence rank) data demonstrating purity, stability, safety, and efficacy under likely conditions of use consistent with the label indication. This construct is applicable to the development and approval of pain drugs for animals. However, there are considerations unique to this therapeutic category across veterinary target species and notably some key differences in the development pathways for companion versus food animals. The challenges of canine osteoarthritis pain drug development as well as the regulatory pathway for bovine pain drug development have been well-described in recent reviews (Sharkey 2013, Smith and Modric 2013).

Although standardized metrics of pain recognition have been formalized for laboratory animal welfare, human perceptions and interpretations of animal pain at the drug development and clinical practice levels remain polymorphic as evidenced by the wide variety of pain measurement schemes and tools developed over the last two decades (Viñuela-Fernández 2007, Rialland et al., 2012, Gebhart et al, 2009, Sharkey 2013). The diversity in companion and food animal pain signaling behaviors within and across breeds and species, is undoubtedly anchored in stimulus-response prey-predator hierarchy and further modulated (confounded) by the human-animal social as well as food animal management and husbandry conditions. Recognizing these fundamental challenges is a first step on the path of animal pain drug development for FDA approval.

Origins of candidate pain drugs

Human drug discovery and/or pre-clinical development programs have fueled the majority of drug pipelines represented in the current listing of FDA approved animal drug applications. Focusing on FDA approved animal pain drugs over the last two decades (excluding pre-anesthetics, generics and sedatives), development efforts have yielded over 20 new animal pain and/or anti-inflammatory application approvals comprised predominantly of non-steroidal anti-inflammatory drugs (NSAID) and opioids (http://www.accessdata.fda.gov/scripts/animaldrugsatfda/). Human pain drug approvals have followed this trend with opioids and NSAIDs representing the majority of FDA pain drug approvals in that same time period (Woolf 2010).

For human and animal pain drugs early steps leading to development typically originate with some knowledge of the molecule class directed to a molecular target, nociception pathway or binding locus from which ideally, clinically-relevant measurable outcomes will corroborate the specific label indication in the target species. For human pain drug development, candidate activity screening progresses to whole animal pre-clinical studies typically conducted in rodents, rabbits and dogs and generally focuses on toxicological and kinetic characterization. Such pre-
clinical screening activity, which may be cost-prohibitive for most animal health companies, can be linked to some high throughput screening engine tasked to serve the human drug pipeline. Hence, animal health companies may license technology or rights to development based on results from studies in this early feasibility stage.

**Early signals of efficacy**

Although early human drug characterization studies in laboratory animals do not have sufficient inferential value to fully support the target veterinary animal label indication (dogs may be the occasional exception), such early phase studies can help define the veterinary drug therapeutic rationale and provide insight to the dose-response relationship, justifying further investigation.

Initial signs of pharmacologic activity used to forecast efficacy responses may be inferred from established structure-activity relationships, ligand binding signaling, and/or whole animal model studies. Early stage studies may also evaluate markers of inflammation or nociception depending on the drug class. However, laboratory animal pre-clinical models in human pain drug feasibility screening may yield results relevant to the laboratory test system but not relevant as predictive outcomes for moving human drugs forward into clinical development and FDA approval (Whiteside et al 2008, Berge 2011). Similarly in animal pain drug development, conclusions derived from early stage non-target species models or surrogate marker laboratory endpoints may not be predictive of success in late stage clinical development; e.g., in multi-site field trial(s) required for FDA approval. It is critical to account for inter-species and laboratory test system differences within and across laboratory pain models before moving into time and cost consuming later stage development in the target veterinary species. The utility of surrogate endpoints or human-relevant biomarkers that are not validated for signal detection in target veterinary species should be critically evaluated for predictive reliability in designing late phase studies for FDA approval.

**Defining label indication**

It is important early in the development process, to establish the proposed label indication for the pain drug. Per FDA regulations, 21 CFR § 514.4(b)(2)(i):

"for a new animal drug intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease over a dosage range, the sponsor must demonstrate by substantial evidence that the new animal drug will be effective for the intended use at the lowest dose of the dosage range suggested in the proposed labeling for that intended use."

It is also essential to recognize that pain as well as other therapeutic drug label indications must reference a disease or painful condition (not just a clinical sign uncoupled from a disease or condition) that is being diagnosed, cured, mitigated, treated or prevented. The label indication can reference target clinical signs; however, the signs must be associated with a recognized disease stated on the label. Label indication key word descriptors such as “treatment of” vs “control of” vs “management of” have significant ramifications on pre-approval study designs. Label indication language is typically established on the basis of the ability of a drug under the
label conditions of use, to either cure (treat) the underlying disease, control the signs of the underlying disease or complement other therapeutic modalities used to manage the disease. Animal pain drugs carry the “control of” indication phrase based on recognizing that the available classes of animal analgesics as currently defined, do not treat or reverse the root pathophysiological cause of the painful condition. Uncoupling study designs and outcome measures during any stage of drug development, from an FDA approvable label indication is a major risk to overall drug development success.

Dosage characterization

FDA CVM Guidance 123 well-describes the dosage characterization relationship to the proposed label indication. Pharmacokinetic and if feasible, pharmacodynamic studies conducted in a relevant target animal test system can yield valuable signals of the dose-response relationship. Early dose-response studies conducted in the target species to range the label dosage may be sufficient to characterize the dosage of the new animal pain drug; however, that dosage will ultimately have to be confirmed as effective and safe in the pivotal clinical field effectiveness and target animal safety studies. The dosage characterization of an animal drug may be derived from laboratory animal studies in human pre-clinical development or from model studies of the target veterinary species.

Ideally the same outcome measures used in dosage characterization should be used in the pivotal clinical field effectiveness studies. If this is not possible, a scientific bridge should be considered to rationalize the dosage characterization outcome measures as relevant to those to be used in the clinical field effectiveness study.

In situations where it is anticipated that pivotal clinical field study conditions may substantially contribute statistical “noise” or “bias” of response assessments, a pilot clinical field effectiveness study enrolling animals with the clinical condition may be considered. In the pilot clinical field study, design features and sample size estimations may be evaluated to improve the pivotal study likelihood of success.

Formulation considerations

Early phase laboratory animal studies are typically performed using non-final drug product formulations which limit the inferential value of the results for FDA approval. An approved drug is actually a finished dosage form drug product containing an active ingredient (drug substance intended to furnish pharmacological activity) in a suitable, stable formulation intended for delivery to the patient according to label directions. The definition of a drug is similarly defined by the FDA for the animal and human drug applications in 21 CFR 314.3.

The physicochemical attributes, source and cost of the drug substance are early factors affecting the decision to move forward with the drug product formulation and pre-scale-up processes. Early pharmacokinetic studies in the target species also influence decisions on the route of administration and can be very useful in evaluating and optimizing the final formulation. FDA Guidance 42 references key FDA guidance documents that describe the specific requirements for the Chemistry, Manufacturing and Controls technical section of a drug application.

Multiple engineering batches of prototype drug product may be created in optimizing formulation performance prior to finalizing the formulation and batch process. Main activities
for formulation selection and development to pre-commercial, clinical batch scale may include: sourcing and qualification of vendors supplying drug substance and excipients, development of the batch formulation process, analytical method development and validation including discriminating and stability-indicating methods, development of impurity profiles and degradation products and appropriate methods as well as setting in process and release tests and specifications. FDA CVM requires that pivotal studies for effectiveness, target animal and human food safety be conducted with final formulation drug product. Furthermore, for pioneer drugs, three pre-approval batches produced at least at 10% of the final commercial market batch size, including all batch process records and process data must be filed with the Chemistry, Manufacturing and Controls technical section. Target animal safety and pivotal field effectiveness studies are typically conducted with supplies from one of the pre-approval drug product clinical batches.

**Target animal safety**

FDA CVM Guidance #185 describes the approach for designing, running and reporting studies to characterize the safety of the drug in normal animals. For both food and companion animals, target animal safety (TAS) studies must be in compliance with 21 CFR 58 GLP requirements and conducted in healthy, laboratory (i.e., test facility-owned) animals of the proposed species for indication. TAS studies are typically conducted as a parallel group design, evaluating the upper end of the 1X label dosage for at least 3 times the duration of the label-defined dosing period and including a zero dose exposure, as well as groups dosed with multiples above the upper end of the 1X label dosage. Drugs intended for lifetime dosing or chronic use indications are typically evaluated in a similar dose group design over a minimum 6-month dosing period. TAS studies involving new chemical entities (i.e., drugs not previously approved in a non-human animal species) require complete necropsy with gross and histopathologic examinations of all animals in all treatment groups.

**Human food safety**

FDA guidance documents 3, 149 and 205 – 208 describe the types of studies and provisions for generating data to satisfy the extensive requirements of this technical section. A summary of the human food safety data package requirements for FDA approval of a pain drug in cattle has been described (Smith and Modric 2013). The elements of the human food safety data package described for cattle are applicable to drugs intended to be administered to any food producing animals. Food animal drugs must cover toxicological characterization, establish intake and exposure factors, identify the target organ for detecting residues, identify the marker residue, determine the safe concentration and define the post-dosing withholding time.

These requirements are not unique to analgesics or anti-inflammatories but apply to all new animal drugs intended for food animal use. Furthermore, the human food safety study battery consumes substantial costs and time that translate into overall increased risk in the development pathway to approval. Market size and return on investment projections are used to justify the substantial investment of costs and time balanced against risks of late-phase failure.

**Clinical field effectiveness**
Clinical field effectiveness studies are required to be conducted in a manner to yield outcomes of high inferential value defined as "the confidence with which the data relating to effectiveness of a new animal drug for an intended use under the conditions tested can be used to conclude that the new animal drug will be effective in the target animal population for the intended use and associated conditions of use suggested in the labeling". The legal requirement for conducting a study to satisfy the definition of “adequate and well controlled” and to meet the “substantial evidence” requirement of the regulations drives the compliance standard for conduct of field effectiveness studies. Good Clinical Practice Guidance (VICH GL9) describes the conditions and compliance standards of clinical field study conduct.

Portability of assumptions from laboratory model studies into large multi-site field studies, where variations in pathophysiological as well as sociological factors introduce noise or bias into response outcomes is a challenge, specifically with drugs evaluated for analgesic efficacy. At least one field effectiveness study of the final drug product formulation is required to be conducted across multiple investigators and study sites and may employ a positive or negative control. This study is typically designed under a single protocol as a randomized, prospective, controlled study. With the exception of the human food safety studies, the clinical field effectiveness study generally consumes the longest time and investment expense in a typical pain drug development program.

Animal pain drug clinical field effectiveness studies may involve 200 to 500+ animals with a larger sample size for a food animal “herd or flock” drug or as defined by statistical projections based on choice of control group. Clinically affected animals diagnosed with the targeted condition from which data on efficacy and safety are derived for product labeling are enrolled across multiple study sites, typically under a single protocol. Clinical field safety data may also be collected for an extended period following the effectiveness assessment time frame. For placebo controlled or standard of care contrasts, statistically significant differences between treatment groups as well as clinical benefits of the investigational drug must be demonstrated. Placebo controlled pain studies are ethically challenging and if conducted, must comply with animal welfare needs. This may be done by use of an escape analgesic to be administered based on patient signal. Preemptive non-conflicting pain relief may be considered; however, such treatment may obscure the ability to detect true treatment related outcomes. Placebo effects in pain studies are well-recognized as bias affecting measurement of valid response outcomes, dampening or defeating the ability to attribute discrete responses to the active treatment group (Turner et al, 1994). Furthermore, specific to pain drugs, the absence of a standardized pain scoring system sufficiently robust for use in a multi-site field efficacy study and with sufficient fidelity to distinguish effects of differing drug classes with differing mechanisms of action remains a challenge. Thus, individually tailored field effectiveness studies are typically designed with an adaptation of published pain scoring scale(s) and objective response measurements specific to the species, operable within the field conditions of use, with measurable clinical outcomes attributed to pharmacologic activity specific to drug class with known (or suspected) mechanism of action.

As an alternative to a placebo control, an FDA approved drug (same label indication and species) may be used as the positive control for non-inferiority comparison (confidence interval conformity) with the unapproved pain drug (FDA CVM Guidance 204). In such cases, the label indication for the unapproved pain drug has the same label indication as the approved positive control drug. In general, sample size requirements are higher in positive controlled non-
inferiority study designs compared to placebo controlled studies, in order to satisfy power requirements for evaluating a lack of difference between treatment groups.

An unapproved pain drug can also be evaluated against a standard of care treatment group, when use of an FDA approved drug is not desirable or otherwise unavailable to use as a positive control. However, the unapproved pain drug must be demonstrated to be statistically and clinically superior in the standard of care comparison.

In summary, the pivotal field effectiveness study should facilitate enrollment of clinically diseased animals meeting specific eligibility criteria. This study should provide outcome measures comprised of a statistically evaluable objective response variable as well as clinically relevant responses evident to the owner-producer and/or veterinarian.

Data requirements for FDA approval

FDA requires all reports and raw data from pivotal studies and manufacturing activities to be submitted for review and determination of adequacy to support a legal basis for drug approval leading to marketing authorization. This requirement is not unique to animal pain drug regulatory submissions but is a major factor driving overall animal drug development quality cost, time, and resource allocations. The FDA electronic data submission portal has markedly improved the efficiency of raw data and report transmittal as well as subsequent CVM responses. The e submission process is especially suited to accommodate the phased (i.e., individual separate technical sections over time) submission and review process currently followed by most drug sponsors.

Pain drug approvals

Excluding anesthetics, the relative proportion of new animal drug applications approved by FDA for pain and/or inflammation ranked by species are: canine>equine>feline>bovine>swine. The therapeutic class ranking in number of approved applications is corticosteroids>NSAIDs>opioids. The ranking of dosage form routes of administration across these applications is: oral>injectable parenteral>transdermal/transmucosal.

Efforts by the FDA Center for Veterinary Medicine, specifically in providing a guidance document for NSAID drug development have helped resolve the 20th century shortage of pain drugs in companion animals. However, only flunixin has been approved as an anti-inflammatory in major species of food-producing animals (cattle and swine). The contrast in the number of pain drug approvals between companion and food animals is likely the result of the extensive investment required to meet the FDA requirements for human food safety. Market factors anchored in production practices versus perceived therapeutic need, balanced against food animal profit margins are a factor in return-on-investment decisions for drug development in this sector. Another potential factor accounting for the relative shortage of approved food animal pain drugs is the lack of systematic and widely used outcome measures of analgesic responses, adapted to production standards and conditions of lairage and husbandry specific to each food producing species. Work should continue in recognizing, publicizing, and reconciling this gap in approved food animal analgesics across producers, veterinarians, academicians and drug sponsors.

Selected references


Guidance for Industry #204. 2013. Active Controls in Studies to Demonstrate Effectiveness of a New Animal Drug for Use in Companion Animals Rockville, MD (US) Center for Veterinary Medicine, Food and Drug Administration
Guidance for Industry #205. 2011. Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Metabolism Study to Determine the Quantity and Identify the Nature of Residues (MRK) VICH GL46. Rockville, MD (US) Center for Veterinary Medicine, Food and Drug Administration

Guidance for Industry #206. 2011. Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Comparative Metabolism Studies in Laboratory Animals VICH GL47 Rockville, MD (US) Center for Veterinary Medicine, Food and Drug Administration

Guidance for Industry #207. 2015. Food-Producing Animals: Marker Residue Depletion Studies to Establish Product Withdrawal Periods VICH GL48(R) Rockville, MD (US) Center for Veterinary Medicine, Food and Drug Administration

Guidance for Industry #208. 2015. Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Validation of Analytical Methods Used in Residue Depletion Studies VICH GL49(R) Rockville, MD (US) Center for Veterinary Medicine, Food and Drug Administration


Smith ER and Modric S. 2013. Regulatory Considerations for the Approval of Analgesic Drugs for Cattle in the United States Vet Clin Food Anim 29: 1–10


Using an outcome-based approach has become common in developing desired competencies in professional education, and veterinary medicine is no exception. In veterinary pharmacology, a need has been identified for consensus recommendations on what we want new veterinary graduates to know about drugs and drug decision-making, particularly given the solo nature of many veterinary pharmacology instructors, not to mention the many hats most academic veterinary pharmacologists wear. To fill this need, the outcomes-based approach is being used to develop recommendations on core competencies in pharmacology and therapeutics for Day One veterinary graduates.

Following the 2012 AAVPT Veterinary Pharmacology Teaching Workshop, a working session at the 17th Biennial in 2013 in Maryland, and some short bursts of activity among interested pharmacologists, a formal Working Group was convened in late-2014. Since that time, the Working Group has met via web-based meeting platforms several times and via electronic asynchronous conversations. Individuals who volunteered for the Working Group members included: Dawn Boothe, Jennifer Buur, Trisha Downling, Tammy Dugas, Marion Ehrich, Walter Hsu, Heather Knych, Lester Mandelker, Richard Martin, Tomas Martin-Jimenez, Lara Maxwell, Katrina Mealey, Paul Mills, Stephen Page, Mark Papich, Louska Schipper, Nicolas Villarino, and Arno Werners. Consensus has so far been reached as to the level of detail to provide, species considered, pharmacokinetic knowledge to expect, and decision to exclude specific drug lists, and we are close to a final version of the list of competencies.

Important aspects of the document will likely be: (1) introductory comments explaining our rationale and suggesting further refinements that may be needed according to teaching institution or jurisdiction, (2) diagrams and visualizations of drug movement in the body and drug decision-making, (3) individual competencies grouped by general topic (such as pharmacodynamics, monitoring therapy, and adverse reactions), (4) cross-references or mapping to AVMA Council on Education, World Organization for Animal Health (OIE), and North American Veterinary Medical Education Consortium (NAVMEC) competencies, and possibly (5) references to Bloom’s taxonomy of learning and (6) suggestions about where in the veterinary curriculum to place a competency (pre-clinical vs. clinical, for example). Excerpts from the latest version of the document will be presented at the Biennial.
INTRODUCTION

Resistance is resistance, right? The first thing to clear up about antimicrobial resistance is the definition of resistance. Clinicians are concerned about clinical resistance, based on clinically applicable breakpoints. Approved veterinary breakpoints are developed and approved by the Clinical and Laboratory Standards Institute Veterinary Antimicrobial Susceptibility Testing Subcommittee (CLSI VAST) based on the following.1

- Clinical outcomes coupled with pathogen MIC distributions
- MIC distributions of wild type isolate collections
- Pharmacokinetic/pharmacodynamic modeling

These breakpoints are intended to give guidance on the probability of an antimicrobial working within the context of a combination of pathogen, antimicrobial, disease, animal species, and specific treatment regimen. Deviation from any of these factors greatly diminishes the predictive value of the breakpoint. It is incredibly important that we understand which breakpoints actually have a reasonable link to antibiotic resistance and which ones don’t.

The second type of “resistance” is related to changes in population profiles of “wild type” susceptibility distributions. Instead of a clinical breakpoint, these are now referred to as an “epidemiological cutoff”. These cutoffs are defined to indicate a change from the original population minimal inhibitory concentration (MIC) distribution, and may indicate the appearance of resistance genes. While the wild type cutoff is a component of a clinical breakpoint, without consideration of the other two components of the CLSI breakpoint process they are not necessarily correlated to clinical response. One of the outcomes of using both epidemiological cutoffs and clinical breakpoints in different monitoring systems may be declaring “resistance” at different MICs. This situation is complicated even further by the potential for resistance genes to be present that are not detected by phenotypic testing.

RESISTANCE CHALLENGES IN HUMAN MEDICINE

This session is about resistance challenges in veterinary medicine, but it helps us to see how challenges in human patients parallels our challenges. Therefore, understanding the resistance challenges in human medicine may inform our interaction with resistance challenges in veterinary medicine.

In 2013, the Centers for Disease Control and Prevention (CDC) released a report describing the major antibiotic resistance threats to human health.2 In this report, the major
threats were classified as threat levels of urgent, serious, and concerning. The urgent threats are high-consequence because of significant risks identified across several criteria. These threats may not be currently widespread but have the potential to become so and require urgent public health attention to identify infections and to limit transmission. The urgent threats are *Clostridium difficile*, carbapenem-resistant Enterobacteriaceae, and drug-resistant *Neisseria gonorrhoeae*. *Clostridium difficile* is included because of the increasing (in both prevalence and severity) incidence of pseudomembranous colitis in patients treated with antimicrobials.

The CDC serious classification includes threats that for various reasons are not considered urgent (e.g., low or declining domestic incidence or reasonable availability of therapeutic agents), but which will worsen and may become urgent without ongoing public health monitoring and prevention activities. These organisms are multidrug-resistant *Acinetobacter*, drug-resistant *Campylobacter*, fluconazole-resistant *Candida*, extended spectrum β-lactamase producing Enterobacteriaceae (ESBLs), vancomycin-resistant *Enterococcus* (VRE), multidrug-resistant *Pseudomonas aeruginosa*, drug-resistant non-typhoidal *Salmonella*, drug-resistant *Salmonella typhi*, drug-resistant *Shigella*, methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae*, and drug-resistant tuberculosis.

The CDC classification of “concerning” includes bacteria for which the threat of antibiotic resistance is low, and/or there are multiple therapeutic options for resistant infections. These bacterial pathogens cause severe illness. Threats in this category require monitoring and in some cases rapid incident or outbreak response. These organisms are vancomycin-resistant *Staphylococcus aureus* (VRSA), erythromycin-resistant Group A *Streptococcus*, and clindamycin-resistant Group B *Streptococcus*.

RESISTANCE CHALLENGES IN VETERINARY MEDICINE CLOSELY MIRROR THOSE IN HUMAN MEDICINE

Weese published a review of antimicrobial resistance issues in companion animals (2008), identifying these major organisms of concern. This would still be a good list for resistance challenges in veterinary medicine.

- *Staphylococcus aureus* and *Staphylococcus pseudintermedius*: both methicillin susceptible and resistant.
- Enterococci: *Enterococcus faecium* and *Enterococcus faecalis*.
- *Streptococci*: *Strep. zooepidemicus* and *Strep. Equi* in horses, *Strep. canis*
- *Escherichia coli*
- *Salmonella*
- *Pseudomonas*

Methicillin-Resistant Staphylococcus aureus

While *Staphylococcus pseudintermedius* is a very real resistance concern in veterinary medicine, methicillin resistant *Staphylococcus aureus* (MRSA) highlights issues of zoonotic interactions in multiple veterinary species. A 2008 review article summarized MRSA occurrence in cattle, dogs, cats, sheep, chickens, horses, rabbits, seals, and psittacine birds. Significant research has demonstrated the potential for exchange of isolates between people and their pets. Kottler, et al., evaluated the prevalence of MRSA in people and pets in the same household. The sample consisted of one human nasal swab and one dog or cat nasal and fecal
swab from 586 households. There was no difference in MRSA prevalence in households with human healthcare workers, veterinary healthcare workers, or without healthcare workers. The occurrence of MRSA in humans was 5.6%, with 3.4% in pets. In 4 of the 586 households (0.7%), the MRSA found in humans was the same strain as that found in the pet.

Faires, et al., evaluated the prevalence of concurrent infection in households where either a person or pet had a diagnosed MRSA colonization. In part 1 of the study, 22 households were identified as having an MRSA infection in a pet (19 dogs and 3 cats). In these households, 10 of 56 humans (17.9%) were also colonized with MRSA. In part 2 of the study, 8 households were identified where humans had MRSA cultures from dermal abscesses. In only 1 of these households was MRSA also isolated from a pet. In almost all cases of co-colonization or infection, the isolates were indistinguishable by PFGE.

O’Mahony, et al., evaluated MRSA isolates from dogs, horses, a cat, a rabbit, and a seal in Ireland along with isolates from 10 caregivers. The PFGE results for the equine MRSA isolates were indistinguishable from the results for those isolates originating from the caregivers for the horses.

Several studies have evaluated risk factors for infection with MRSA in companion animals. Faires, et al., evaluated risk factors for 40 MRSA infected dogs compared with 80 MSSA infected dogs. The highest prevalence of both infections was in ears and skin. The statistically significant risk factors for MRSA infection as compared to MSSA infection included the use of any antimicrobial prior to diagnosis (odds ratio 2.84), use of fluoroquinolones (OR 3.58), use of β-lactams (OR 3.58), or intravenous catheterization (OR 3.72).

A retrospective study in horses in Canadian and American referral hospitals evaluated MRSA infections in 115 horses. The infections originated both in the referral hospitals and in the community, with the frequency of both being approximately equal. Community acquired infections were significantly associated with previous hospitalization and previous gentamicin therapy. Hospital-acquired MRSA infections were significantly associated with infected incision sites.

Increasing attention in the literature has been paid to MRSA in swine and potential zoonotic concerns. While swine workers and veterinarians have been demonstrated to have nasal carriage of the MRSA type found in swine herds, epidemiological studies suggest that colonization is primarily limited to those working with the swine and further transmission is limited to familial communities of these exposed workers. In the U.S., the human community-acquired outbreak strains are different from animal strains. In the Netherlands, a type of MRSA (ST 398) is epidemiologically associated with pig and cattle farmers and is said to be > 20% of carriage in humans. MRSA has also been identified in bovine mastitis isolates. The authors of a 2012 study using single nucleotide polymorphisms (SNPs) to evaluate 89 CC398 MRSA isolates proposed that this MRSA originated in humans as a methicillin-susceptible isolate and then acquired tetracycline and methicillin resistance in livestock, but also lost phage-carried human virulence genes. MRSA CC398 has been documented to cause disease in humans, although it is not a major player in MRSA-associated disease in humans and appears to be a poor long-term colonizer.
**Carbapenemase-Producing Enterobacteriaceae**

MRSA is an example of a resistant organism (which may also be multi-drug resistant) that brings the issue of treating our veterinary patients together with concerns about the effect of this pathogen’s presence on our clients. A more recently emerging issue of shared resistance between human and animal pathogens is that of carbapenemase-producing enterobacteriaceae (CRE).

Human cases of CRE have been in the news lately where multidrug resistant pathogens have displayed resistance to this class of antimicrobials previously considered to be omnipotent. And, once again, the situation in veterinary medicine is mirroring the antimicrobial resistance challenges in human medicine. Carbapenemase-producing isolates of *Klebsiella pneumoniae* and *E. coli* have been identified in dogs from a single hospital in Germany, with the clonal nature of the isolates suggesting nosocomial spread. Carbapenem producing E. coli have also been confirmed in clinical isolates derived from dogs and cats in the United States. An analysis of the literature related to potential sources of these organisms cites detection in dairy cows (France), horses (Belgium), a wild raptor (Germany), poultry and swine (China), dogs and cats (Germany and USA), and multiple instances in water and sewage throughout the world. The pattern reasonably supports a hypothesis of spread to multiple veterinary species through environmental dissemination of human sources; this is further supported by no labeled carbapenems for food animals, a cost structure which makes extralabel use in food animals highly unlikely, and limited use in companion animals. While certainly not yet ubiquitous in occurrence, confirmation of these isolates in veterinary species strongly supports the need for continued evaluation of our use of carbapenems in veterinary species, and the potential for the occurrence of these organisms in clinical practice.

**Bovine Respiratory Disease Pathogens – An example of looking to the future of therapy in food animals**

Lubbers and Hanzlicek published a retrospective analysis of Mannheimia haemolytica susceptibility results during 2009-2011 from the Kansas State Diagnostic Laboratory. The percentage of isolates showing resistance to at least 3 of our main classes of antibiotics used for BRD were 42%, 46%, and 63% in 2009, 2010, and 2011 respectively. These data represent diagnostic laboratory isolates primarily from cattle of types associated with high risk for respiratory disease and do not represent a random sample across all cattle. However, these data represent isolates from cattle where the attending veterinarian felt the need to submit samples for susceptibility testing, and the trend in multidrug resistance in this population of cattle is concerning. While the data from the paper encompass 2009-2011, Dr. Lubbers provided this update through 2013.
In this figure, the X axis is the number of antimicrobials to which the isolate is resistant. The Y axis is the percent of *Mannheimia haemolytica* isolates. The 5 antibiotics represented are florfenicol, spectinomycin, enrofloxacin, tilmicosin, and oxytetracycline. Ceftiofur consistently tests as susceptible; however, that breakpoint is set at 2 µg/ml and the MIC90 of the wildtype population is typically around 0.03 to 0.06 µg/ml, requiring at least a 7 dilution jump to be called intermediate. Tilmicosin results closely agree with tulathromycin results. Of 179 M. haem isolates in 2011, 152 (85%) were direct matches, 14 were susceptible for tulathromycin and intermediate for tilmicosin, 10 isolates displayed resistance for one and intermediate for the other. The difference between 4 and 5 resistance findings is typically absence or presence of resistance to florfenicol.

When looking back at single drug trends in *Mannheimia haemolytica* at the Kansas State Diagnostic Laboratory a consistent trend is evident from 2005-2014.
The top dilution tested for tilmicosin up through mid-2007 was 32 µg/ml, making the maximum concentration on the chart ≥ 64 µg/ml. In mid 2007, a concentration of 64 µg/ml was added, making the top concentration on the chart >64 µg/ml.
Summary

Many of the resistance concerns in veterinary medicine mirror the challenges in human medicine. While it is possible for the discussion to devolve into one of assigning blame to antimicrobial use practices in one arena or the other, the solution is to adopt well-designed antimicrobial use strategies for all applications of antimicrobials.

ANTIMICROBIAL THERAPY IN COMPANION ANIMALS

Paul Morely
Colorado State University
Historically, the focus of evaluating the pharmacodynamics of anticancer drugs has been on observed toxicity. This is not to say that efficacy has been ignored, but efficacy has been evaluated in the context of how well a drug may work at the dose producing “tolerable” toxicity. Dose determinations for the traditional cytotoxic agents have not been based on maximum efficacy. Instead, Phase I trials of cytotoxic agents aim to identify a maximum tolerated dose (MTD) which is the highest dose level at which ≤ 33% of patients will experience a dose-limiting toxicity (DLT). Furthermore, adjustments in dose during the course of treatment are not based on anti-tumor response but degree of toxicity, with dose-increases rarely ever made if toxicity is not observed. With regard to measurements of efficacy, standardized criteria are available for evaluating efficacy of therapeutic regimens against solid tumors and lymphoma and these have been used directly or adapted for veterinary medicine but it is the standardized criteria for evaluating toxicity that determine dosing adjustments in the vast majority of cases. This is because of the relationship between dose-toxicity and dose-efficacy curves for cytotoxic chemotherapy where both are assumed to move in parallel as dose increases or decreases. This is supported by studies showing that canine lymphoma patients experiencing Grade III or IV toxicity or requiring dose reductions due to toxicity have a longer first remission. However, the ability to dose to a pre-determined toxicity has not been possible because of the inability to predict a specific level of toxicity with a given dose.

One of the major challenges in veterinary oncology is the large degree of interpatient variability which is expressed not only as differences in the severity and/or type of toxicity but also differences in effectiveness in populations of pets administered the same dose of drug. Human oncology has identified pharmacogenetic variability as a significant contributor to the differences in treatment outcome. Genetic polymorphisms in drug transport proteins and Phase I/II metabolizing enzymes have been identified in human populations to impact the disposition of numerous chemotherapy agents and thus contribute to the variability in outcomes. While this field is growing in veterinary medicine and has made some important contributions to the understanding of PK/PD variability with some cytotoxic agents, there is still much to learn. A more thorough understanding of the interrelationship between pharmacogenomics, pharmacokinetics and pharmacodynamic actions of anticancer drugs may allow for the implementation of more patient-tailored anticancer therapy in veterinary medicine.

Recently, an improved understanding of the biology of cancer and the identification of molecular drivers for a few cancers has shifted some of the focus from traditional cytotoxic agents to molecularly targeted agents. One class of these agents is the receptor tyrosine kinase inhibitors (RTK) which function by blocking the activation of cell surface receptors and prevent signaling through survival and proliferation pathways. With the recent approval/conditional approval of two of these agents in veterinary medicine a shift in the way that therapeutic effect is measured may need to be considered. As is the case in human oncology, there is also a need to identify the
most rational uses for these drugs and identify the best methods for combinations with traditional cytotoxic drugs. Although these targeted therapies are still often dosed at MTD, there is some evidence that this may not be necessary and identification of a biologically effective dose (BED) may allow for the use of doses below MTD. The identification of a BED presents some obstacles as this may require more invasive techniques to answer critical questions:

- Why do some patients experience toxicity while others do not?
  - Differences in metabolism/drug exposure?
  - Polymorphisms or differential expression of target in normal tissues?
- Is drug distributing to the tumor tissue
  - Can imaging studies be utilized to identify drug distribution?
  - Is tumor accessible for biopsy and analysis by LC-MS/MS?
- Can we demonstrate a molecular effect in the tumor tissue?
  - Demonstrating proof of mechanism (i.e. target engagement or inhibition).
  - Are drug concentrations required for demonstration of effect in vitro achievable in vivo?
- Does target inhibition lead to a clinical effect/response
  - Demonstrating proof of concept (i.e. reduction in activation of RTK leading to tumor shrinkage, prolonged remission, or improved sensitivity to cytotoxic chemotherapy).
- Can we identify the same effect in another tissue that is easily accessible and are there correlations that might allow that tissue to act as a surrogate marker for the tumor?
  - Is the target expressed in normal tissues?
  - Does activity depend on a mutation of the target which may not be present in normal tissues?
  - Are the surrogate tissue and the tumor tissue exposed to similar drug concentrations?

The relationships between drug exposure, target modulation and antitumor activity are extremely important concepts to understand in order to maximize therapeutic success. Veterinary oncology provided the first study, in any species, to show these relationships following the use of a targeted agent (Toceranib) for treatment of canine mast cell tumors. Similar studies in veterinary oncology have also been used to evaluate in vitro and in vivo relationships in drug combinations that pair a cytotoxic agent with other drugs targeting biologic processes such as autophagy and epigenetic histone modifications.

The issue of identifying biomarkers and developing assays that can be validated and qualified forms a large area of current research in human oncology and similar work in veterinary oncology may provide a mechanism by which the treatment of cancer in pet animals can be improved.

References:


Proper assessment of oncologic outcome is a critical step in determining efficacy of a novel agent or protocol in veterinary cancer. Outcome assessment is complicated by a lack of standardization in response criteria and, perhaps most relevantly in the era of targeted therapy, a lack of standardization for what constitutes stable disease. Again, in the era of targeted therapy, correlative pharmacokinetic and pharmacodynamic assessments are critical to determine a cause for lack of response.

**Conventional Response Assessments**

With traditional cytotoxic chemotherapeutics, response criteria are fairly straightforward as tumor diameter and/or volume are used to assess response according to several published methodologies (e.g., the VCOG RECIST criteria; Table 1) (Nguyen et al., 2013). Both of these assessment schemes have benefits and limitations regarding accuracy/reproducibility of measurement and clinical relevance. Timing of response assessment, and durability of responses are other factors that vary between studies and make direct comparisons challenging. For example, recent registration trials have used overall response rate at 12 weeks as their primary endpoint (Vail et al., 2012), while other studies with the same agents have used best overall response rate at any time as their endpoint (Rassnick et al., 1999; Rivera et al., 2013).

**Table 1: Definition of best response according to WHO and Veterinary Cooperative Oncology Group RECIST criteria**

<table>
<thead>
<tr>
<th>Term</th>
<th>WHO Definition</th>
<th>RECIST Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Response (CR)</td>
<td>Disappearance of all target lesions.</td>
<td>Disappearance of all target lesions.</td>
</tr>
<tr>
<td>Partial Response (PR)</td>
<td>&gt;50% reduction in the sum of the maximal volumes of target lesions (vs. baseline)</td>
<td>&gt;30% reduction in the sum of the maximal diameters of target lesions (vs. baseline)</td>
</tr>
<tr>
<td>Progressive Disease (PD)</td>
<td>&gt;25% increase in the sum of maximal volumes of target lesions (vs. baseline), or the appearance of new lesions</td>
<td>&gt;20% increase in the sum of maximal diameters of target lesions (vs. baseline), or the appearance of new lesions</td>
</tr>
<tr>
<td>Stable Disease (SD)</td>
<td>&lt;50% decrease or &lt;25% increase in sum of maximal tumor volumes (vs. baseline)</td>
<td>&lt;30% decrease or &lt;20% increase in sum of maximal tumor diameters (vs. baseline)</td>
</tr>
</tbody>
</table>

WHO = World Health Organization.  RECIST = Response Evaluation Criteria in Solid Tumors
It is readily evident that such criteria may not be appropriate for the newer targeted agents that are more likely to be cytostatic than cytotoxic and result in stabilization of disease rather than measurable regression. In such cases, it is critical that a designation of “stable disease” persist for a clinically meaningful period of time, generally not less than 6-8 weeks, in order to not simply reflect slow progression rather than a true effect of the novel therapy. Furthermore, the true assessment of disease stabilization as a relevant endpoint is challenging. Novel phase-2 study designs such as the randomized discontinuation trial (RDT) have been developed to better evaluate this increasingly important endpoint (Rosner et al., 2002; Stadler, 2007; Stadler et al., 2005).

Trials that evaluate cytostatic agents in tumors with a variable natural history seem ideally suited for RDTs as the “no treatment effect” is hard to control for in these cases. In essence, these trials serve to enrich and homogenize for those patients that are likely to benefit from the static-agent. RDTs involve a 2-stage trial design (Figure 1) where the first stage involves a “run-in” phase where all patients receive the cytostatic agent under investigation. At the end of the run-in phase, assessment of disease response is made. If a response is noted, the subject continues on with the investigational drug, while if progression (or excess toxicity) is noted the subject is removed. Those patients that meet stable disease criteria enter the second stage of the RDT and are randomized to either continue with the investigational drug or placebo (the discontinuation arm). Then at predetermined times, follow-up determinations are made. Endpoints in stage 2 of the trial at these follow-up intervals are “stable or better” versus “progression”; that is, progression-free rate. Time to event measures could be applied as well (e.g., time to progression), although this takes more time to complete. If a subject progresses in the second stage, the code can be broken and if in the placebo group, the investigational drug can be reinstituted.

Response in the Era of Targeted Therapy
A major shift in cancer drug development, both in human and veterinary medicine, concerns the change from “traditional” cytotoxic agents to novel, “targeted” agents (Booth et al., 2003). Whereas there is generally a clear relationship between dose and effectiveness for most cytotoxic agents, this may not be the case for many targeted agents. This represents a switch from a primary focus on toxicity to one of identifying a dose that optimally inhibits a specific target.
(Kummar et al., 2006) in phase-I studies; that is, the biologically effective dose (BED) may not equate with the maximally tolerated dose (MTD), a dose that is the more traditional “working dose” for efficacy trials. Establishment of this BED may be more likely based on achievement of a target drug exposure or inhibition of a specific molecular target in tumor or surrogate tissue, rather than objective tumor shrinkage.

**Early Predictors of Response**

Early predictors of response would be very useful in patients, to minimize cost, time and potential toxicity associated with the administration of a drug that will likely not be efficacious. When a drug target is well established, can evaluation of changes in this drug target in tumor or surrogate tissues be utilized as an early response predictor? This has been incompletely studied in both humans and dogs, likely as a result of challenges with serial biopsy acquisition compliance on the human side. Encouragingly, early studies with toceranib phosphate (Palladia®, Zoetis) in dogs with mast cell tumor did suggest that inhibition of phosphorylation of the KIT receptor tyrosine kinase was associated with a higher likelihood of clinical response (Pryer et al, 2003). Additional, less specific changes, such as alterations in biomarkers of tumor cell proliferation or apoptosis, could be used as predictors of response to a variety of both classical cytotoxic as well as targeted therapies.

More useful and better studied on the human side is the use of potential imaging-based endpoints for the prediction of eventual response to therapy. Functional imaging, such as PET-CT, has been best studied for these types of predictions. Optimally, changes in functional imaging parameters should be corroborated by changes in target inhibition or tumor cell behavior. This was elegantly demonstrated in dogs with lymphoma by Lawrence et al (2009), where the measurements of fluorothymidine uptake in tumors (a measure of proliferation) via PET-CT was positively correlated with both clinical response to the novel agent GS-9219 (rabacfosadine, Tanovea®, VetDC) as well as reductions in tumor cell proliferation as assessed by Ki67 immunohistochemistry.

**Conclusions**

In conclusion, further moves toward standardization of response criteria, no only in terms of target lesion measurement but in terms of timing of assessment and duration or responses / stable disease to be “clinically meaningful” will facilitate clinical trials with novel agents moving forward. In the clinic, novel early methods to predict response will greatly improve the utilization of the novel agents that are becoming increasingly available in veterinary oncology.

**References**


ANALYZING DRUG EFFECTS IN CYTOTOXIC VERSUS TARGETED ANTICANCER THERAPIES: IN VITRO AND IN VIVO TRANSLATION

Daniel Gustafson
Colorado State University
An introduction to the US Pharmacopeial Convention

Dawn Boothe, Carol Davis, Gigi Davidson

Founded in 1820, the U.S. Pharmacopeial Convention (USP) is a scientific nonprofit organization that sets standards for the identity, strength, quality, and purity of medicines, compounded preparations, food ingredients, and dietary supplements manufactured, distributed and consumed worldwide. USP’s drug standards are specified in the adulteration and misbranding provisions of the Federal Food, Drug, and Cosmetic Act and apply to all medicines in the US including those manufactured or compounded for veterinary patients. USP’s drug standards are enforceable in the United States by the Food and Drug Administration, state regulatory boards, and in 140 countries worldwide. This presentation will provide insight into USP's history, mission, strategic plan, standards-setting activities, and relevance to the practice of veterinary medicine.
Individualized drug therapy increasingly is being recognized as an important aspect of health care for both human and veterinary medicine. The lack of commercially available drug formulations often leads the veterinarian to prescribe or dispense a product specifically designed and compounded for their patients’ medical needs. Compounding has been defined by the National Association of Boards of Pharmacy (Model State Pharmacy Act) as the preparation, mixing, assembling, packaging, or labeling of a drug or device, as the result of a practitioner’s prescription drug order (or initiative) and based on the practitioner/patient/pharmacist relationship [http://www.iacprx.org/index.html, accessed July 2004]. In 1997, the US Supreme Court defined drug compounding as “a process by which a pharmacist or doctor combines, mixes, or alters ingredients to create a medication tailored to the needs of an individual patient.” [Emphasis Id. at 361].

**Historical perspective**

Compounding is as old as drug use. A major advent in the profession of pharmacy and the science of compounding was the development of drug standards. In the 19th century, the United States Pharmacopeia (USP) began its role in the provision of drug standards, thus assuring strength and purity of drug materials. It maintains this often unrecognized, yet critically important role today; its pharmacopeia (United States Pharmacopoeia/National Formulary; USP/NF) are the legal standards recognized by the Food and Drug Administration.  

Compounded products predominated into the twentieth century; as late as the 1930s and 40s, 50 to 60% of human drugs were compounded by pharmacists. However, in the late 19th century, the need for new, therapeutically useful compounds led to the advent of pharmaceutical research, and shortly thereafter, pharmaceutical manufacturing. By the 1950’s, advances in manufacturing technology led to the mass production of drugs, causing pharmacists to largely become dispensers, rather than compounders, of drugs. The 1980’s and 90’s were accompanied by a resurgence in compounding in human medicine for a variety of reasons such that by the late 1990’s, 43,000 human drugs, or 1% of dispensed human drugs, are compounded each day by approximately 10% of human pharmacies. The history of veterinary compounding has paralleled human compounding. The cost of approval of an animal drug surpasses $15-20 million. The economic return of animal drug approval not surprisingly is low (generally well below $100 million); subsequently, the financial incentive to pursue animal drug approval compared to human pharmaceuticals is much less. Further, because of cost differences, veterinarians often will prescribe a human or human generic drug. Despite the fact that it should not be, compounded preparations are often prescribed because they can be cheaper. The issues with veterinary compounding include are not necessarily encountered in human compounding. Unfortunately, animal care givers, veterinarians and pharmacists often are unaware of these differences.

**Definition and Regulations for Compounding**

The regulatory philosophy of the FDA toward veterinary and human drug compounding differs, potentially leading to confusion and misinterpretation of regulations. Failure of either
veterinarians or pharmacists to appreciate and address these differences can contribute to inappropriate compounding.

**The Food and Drug Administration**

The advent of pharmaceutical manufacturing in the early 1900s increased human exposure to drugs, and thus the risk of adverse drug events. In response to this increased risk, in 1908, Congress enacted the Federal Foods and Drug Act. This act provided for the formation of what eventually became the FDA, and its regulatory powers. Regulatory actions of the FDA are delineated in Congressionally approved acts or their amendments. Thus, an act of Congress literally is necessary for the empowerment of the FDA with its regulatory actions. The regulations ("rules") established for implementation of the FDCA and its subsequent amendments are published in codified form in the Code of Federal Regulations (CFR), which is available for public review. To facilitate understanding of the regulations by FDA staff, and to a lesser degree, industry and the public, the FDA may publish Compliance Policy Guides (CPG) for each set of regulations. The CPG direct FDA regulatory actions. However, in contrast to an Act or its regulations, which are legal documents, CPG are not legally binding, and are open to interpretation by the FDA. Indeed, the current CPG for compounding in animals (CPG 608.40) specifically state that the "guidance describes FDA’s current thinking on what types of compounding might be subject to enforcement action". Because CPG represent current interpretation of the laws by the FDA, they can be altered without public comment by the FDA (as has recently happened for veterinary compounding) as it deems necessary to remain in compliance with the law. As a result, FDA regulatory guidelines are "moving targets," contributing to difficulty in anticipating which activities might or might not be regulated by the FDA.

In the late 1930’s, over 100 persons died after being treated with sulfanilamide prepared in a toxic vehicle. The resultant public outcry was instrumental in the passage of the Food, Drugs and Cosmetic Act (FDCA). With passage of the FDCA, as (manufactured) drugs increasingly were administered to humans, the FDA focused its initial activities toward drug safety. However, because the law was intended to regulate the emerging pharmaceutical industry, and because compounding had, up to that time, proven vital to effective drug therapy, the act was not intended, nor was it interpreted by the FDA, to either allow or prohibit the compounding of drugs. In 1962, the FDCA was amended to include the assurance of drug efficacy in the mandated activities of the FDA. Again, compounding was not specifically addressed; further, animal drugs were not addressed. It was not until 1968, with amendment of the FDCA by the Animal Drug Amendment, that animal drugs were distinguished from human drugs. This amendment provided for the formation of the Bureau (later renamed to Center) of Veterinary Medicine (CVM) within the FDA. The mission of the CVM, as mandated by Congress, was (is) assurance of both animal and public health resulting from drug use in animals. Thus, although the regulatory activities of the CVM clearly focus on animal drug efficacy and safety, a major proportion of their actions address the impact of animal drug use on human health (eg, unsafe animal residues). Often, this mandated focus on public safety may lead to prioritization of issues related to food animal or public health over issues related to companion animal health.

**Compounding of Human Drugs**

Compounding of human drugs was not specifically addressed in either the original FDCA or its 1962 amendment. However, the FDA is empowered to regulate any drug (or any product intended to be used as a drug) and interprets a compounded drug to be an unapproved, new
As compounding increased toward the end of the 20th century, FDA regulation of the human drug compounding was specifically addressed in 1997 with passage of the Food and Drug Administration Modernization Act (FDAMA). This Act, which does not apply to veterinary medicine (compounding of animal drugs addressed below), included Section (503A) entitled “Pharmacy Compounding”. Among other things, FDAMA was intended to specifically legalize certain aspects of human compounding, including compounding from selected bulk substances (defined below). However, in order to protect consumers, the act also attempted to provide the FDA with criteria by which inappropriate compounding could be identified and subsequently regulated. The intent of FDAMA was “to ensure continued availability of compounded drug products as a component of individualized therapy, while limiting the scope of compounding so as to prevent manufacturing under the guise of compounding.”

These included the amount of drug product compounded in anticipation of need, whether or not the compounding of the drug was individual-patient driven, and, because it was perceived by the FDA as an indication of manufacturing of inappropriate amounts of a compounded drug, the Pharmacy Compounding Section of FDAMA prohibited the advertisement of compounded products. However, this aspect of the law was subsequently challenged by the pharmacy profession, based on infringement of the second amendment (right of free speech). The Federal courts agreed, but stipulated that this portion of the law could be severed from the remaining portions of the law. A higher court indicated that the portions of the law could not be severed and the US Supreme Court agreed to hear only the challenge to the second amendment. Ultimately, the US Supreme Court agreed that the restrictions on advertising did infringe on second amendment rights, and because the advertisement portions of the laws could not be easily separated from the remainder of the law, the entire Pharmacy Compounding section of FDAMA was invalidated. Ironically, with the invalidation of the Pharmacy Compounding section of FDAMA, while gaining the right to advertise their expertise, pharmacists have subsequently failed to gain protection of their right to compound human drugs (Gibbs 2002), including compounding from bulk substances. Despite the lack of legal protection, the CPG for FDAMA as currently written do indicate tolerance of the FDA toward compounding from bulk substances if an approved version (human) of the bulk substance exists.

**Compounding of Veterinary Drugs**

In contrast to compounding of human drugs, federal regulation of veterinary compounded veterinary drugs is specifically and legally addressed by the Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994. As the animal counterpart to FDAMA, it amends the FDCA. The major benefit of this act to the veterinary profession was legalization of the already common practice of extra-label drug use (ELDU) in animals [(Sec 512 (a) (4)], as long as the conditions stipulated in the regulations are met. Regulations relevant to ELDU have been delineated for veterinarians by the AVMA in a brochure (Veterinary Therapeutics; June 2003) and a user-friendly algorithm (summarized in Table 1). Veterinary ELDU is legalized by AMDUCA only for approved (human or animal) drugs and not for products intended for use as drugs but are not approved drugs. Such substances are perceived by the FDA to be (unapproved) drugs, and as such, fall under their regulatory jurisdiction. This includes novel ingredients such as herbs and nutraceutical, as well as products compounded outside the stipulations of AMDUCA.

Compounding of animal drugs is specifically legalized by AMDUCA (21 C.F.R Section 530). However, the compounding must be implemented in accordance with the relevant provisions of ELDU (Table 1). As such, AMDUCA stipulates that compounding must be
performed by either a licensed veterinarian or pharmacist (thus assuring the rights of both professions to compound) in the context of a valid veterinary client patient relationship and that no approved dosing form or concentration of the drug (human or animal) commercially exists for the treatment of the diagnosed condition.

The interpretation and implementation of compounding regulations of AMDUCA are delineated in CPG 608.400: Compounding of Drugs for Use in Animals. The original CPG (written in 1996) for compounding regulations included the FDA’s working definition of compounding: “any manipulation of a drug product to produce a dosage form drug other than that provided for by the labeled directions for use of the approved drug product.” However, because CPGs are not legal documents, the inclusion of a definition was considered by the FDA to be misleading, and as such, the definition is not included in the current version of the CPG. Rather, the updated CPG describes those activities not considered to be compounding. These include mixing, reconstituting or other acts [on the drug] that are performed in accordance with the approved labeling provided by the manufacturer. Thus, any modification in the finished dosing form of the approved drug that is not specifically delineated on the drug label (which includes its accompanying package inserts) is considered as compounding. This includes modifications as simple as dilution beyond that stipulated on the label, or crushing a tablet to be prepared in syrup, or the combination of two or more finished drug products in the same preparation. The FDA considers any compounded product (human or animal) to be a new, finished (that is, ready for administration) drug, and, because it undergoes no federally-mandated approval process, an unapproved drug. The FDA assumes that both public and animal health potentially are put at risk if compounded drugs are administered to veterinary patients since the drugs are not accompanied by “adequate and well controlled safety and effectiveness data,” particularly if not compounded in “adherence with pharmaceutical chemistry and current good manufacturing practices” (CPG Section 608.400). The FDA anticipates that compounded products may cause adverse reactions or contain potentially harmful excipients, and that the unscientific assignment of withdrawal times to compounded food animal products may lead to potentially harmful tissue residues. Accordingly, the laws (eg, AMDUCA), regulations and CPG that address compounding of animal drugs focus (although not exclusively) on protection of human (public health) safety. The original CPG also included directions as to how compounding should be performed, but these also are absent in the updated CPG because regulating compounding to such an extent is outside the authority of a federal agency. It is the intent of the FDA to defer to state authorities for such regulations.

Several sources of active ingredients are used for compounded animal drugs. Legal sources are limited to FDA-approved finished forms of either animal or human drugs; the FDA makes no distinction as to which (animal versus human) is the preferred source. Because no other source is legalized, all other sources are considered by the FDA to be illegal, including non-FDA approved finished drug products obtained outside of the United States and bulk substances. A bulk drug substance is legally defined [21 CFR 207.3(a)(4)] for both human and animals as “any substance that is represented for use in a drug and that, when used in the manufacturing, processing or packaging of a drug, becomes an active ingredient or a finished dosage form of the drug.” In laymen’s terms, any drug or drug preparation ingredient not prepared in a finished dosage form is considered to be a bulk substance. Whereas AMDUCA regulations specifically state that ELDU of drugs compounded from an approved animal or human drug is permitted, (21 C.F.R Section 530), it further states that “nothing (in [Part 530]) shall be construed as permitting compounding from bulk drugs.” This statement emphasizes that
the law and its regulations do not address compounding from bulk drugs (i.e., compounding from drugs is not legalized and thus, according to the FDA, is illegal). It was included in the law, in part, because compounding from bulk substances is perceived by the FDA to place humans at an increased risk to inappropriate drug residues. It is this statement that is the focus of challenge by the pharmacy profession as it seeks Congressional action to change the FDA’s interpretation of compounding from bulk substances in animals.

Confusion has surrounded the issue of compounding of animal drugs; this reflects, in a part, wording of the law. However, interpreting what is legal and what is not was complicated by the original CPG for the compounding aspects of AMDUCA. These were written (in 1996) prior to, and in anticipation of, the regulations themselves. Consequently, the CPG, which represented “current thinking” of the FDA, did not necessarily represent the final regulations of the law. Despite the fact that AMDUCA specifically indicates that compounding from bulk substances is not addressed (and thus, not allowed), the 1996 CPG implied a prioritization of FDA-CVM regulatory action toward compounding from bulk substances. The CPG indicated that normally, regulatory action would not be pursued for compounding from bulk substances in small animals if an approved finished version of the bulk substance existed. This contradictory guideline was misinterpreted to mean compounding from bulk substances was legal, leading to two negative sequelae. First, compounders abused (either intentionally or not) the “tolerant” approach of the FDA toward compounding from bulk substances in non-food animals; abuses ranged from compounding for food animals to compounding for non-food animals, but outside the stipulations of AMDUCA. Further, the original CPG compromised the FDA’s ability to regulate inappropriate compounding: the perception of tolerance complicated the FDA’s ability to prove abuse. As such, in 2003, the CPG for compounding regulations of AMDUCA were updated to remove any implied prioritization of regulatory action or an attitude of toleration toward compounding from bulk substances, thus bringing the FDA into compliance with the law. As mandated by the FDA, the updated CPG for compounding of veterinary drugs also is in harmony with those written for FADMA, the human counterpart to AMDUCA (CFR 503A).

The FDA specifically states in its 2003 CPG for animal drug compounding that the updated CPG do not apply to compounding of products if either an approved animal or human drugs is used as the source, as long as such compounding adheres to conditions stipulated by AMDUCA (because such action is legal, regulation is not indicated and no CPG are needed). Rather, the updated CPG focus on the compounding of unapproved new animal drugs (those compounded outside of AMDUCA) “in a manner that is clearly outside the bounds of traditional pharmacy practice.” In contrast to the 1996 CPG, the 2003 CPG specifically state that AMDUCA does not permit veterinarians to compound unapproved, finished drug products from bulk drug substances, unless the finished drug is not a new animal drug. Because any compounded animal drug is a new (yet unapproved), animal drug, then no circumstances exist in which compounding from bulk substances is allowed (except for bulk substances delineated in Appendix A of the CPG; see later discussion). It is this aspect of the law that has caused many distributors or suppliers of bulk drug distributors to decline their sale to veterinarians or pharmacists because of their concern over regulatory actions by the FDA.

Despite the changes in the 2003 CPG, the FDA has indicated verbally that their intent with the new CPG is not to alter their regulatory priorities, but rather to facilitate their regulatory abilities. Regulatory action by the FDA will focus, according to the CPG, on “compounding that is intended to circumvent the drug approval process and provide for the mass marketing of products that have been produced with little or no quality control or manufacturing
standards to ensure the purity, potency and stability of the product.” The implied prioritization or
tolerance to compounding from bulk substances of the 1996 CPG has been replaced in the 2003
CPG with a delineation of thirteen compounding actions which will be considered for regulatory
action by the CVM. This list is not inclusive, and can be modified as needed by the CVM to
remain in compliance with the law and to protect public safety. The following actions are listed
(not necessarily in order of regulatory priority). In general, violations that may result in harm to
public health (e.g., involves compounding for food animals) are most likely to be regulated,
followed by compounding that may harm animal health.  Regulatory action toward veterinary
compounding is more likely to occur if: 1. The health of the animal being treated with the
compounded drug is not threatened and if suffering or death is not likely to result from failure to
treat with the compounded product. 2. Compounding is done in anticipation of prescriptions,
unless in limited amounts as indicated by a prescription issued in the confines of a
veterinary-client-patient-relationship. 3. Compounding is performed using drugs prohibited for
extra-label use in either food or non-food producing (currently, no drugs are prohibited for use in
non-food producing animals but this clause allows for action should such drugs exist). 4.
Compounding occurs from drugs with a restricted distribution system (drugs whose use is
restricted by the FDA, such as thalidomide). 5. Compounding occurs from drugs that are not
approved (human or animal, including bulk drugs) unless the product is specifically addressed
for regulatory discretion by the FDA in Appendix A (see below). 6. Compounding involves the
use of commercial scale manufacturing equipment (implying the manufacture of large amounts
of drug products, in anticipation of need, and thus not patient driven). 7. Compounding occurs
for third parties with subsequent resell to individual patients (indicating that resale by a
veterinarian to a client of a product compounded by a pharmacist is subject to regulatory action)
or compounded products are offered at wholesale with intent to resale (the product is then
considered an unapproved, manufactured drug). Few veterinarians or pharmacists realize that
resale of compounded products is illegal. However, some State Boards of Pharmacy allow “for
office use” products which are intended for short-term dispensing to animals (clients) when
prescription availability is precluded (e.g., weekends or evenings). 8. Compounding is not in
compliance with applicable state pharmacy laws. 9. Compounding results in piracy, that is, the
compounded label does not contain sufficient information as delineated in AMDUCA regulations
(including withdrawal times, name and address of the prescribing veterinarian, name of the active ingredients, directions for use, cautionary statements, and veterinary-specified withdrawal time). The remaining 3 guides relate to food animals, including
the use of human drugs, avoidance of drug residues and scientific establishment of withdrawal
times. Veterinarians should avoid as much as possible those pharmacies whose practices in compounding clearly are in violation of the law.

In contrast to the CPG for animal compounding, the CPG for FADAMA state that compounding of human drugs from bulk substances will be tolerated as long as an approved finished version of the drug exists. Because pharmacists may not be aware of regulatory differences between animal and human drugs (and do not realize that CPG are not legally binding), pharmacists may assume that compounding of animal drugs from bulk substances is legal as long as an approved animal version of the drug of interest exits.

Drug Quality and Security Act (DQSA)

In 2012, an outbreak of fungal meningitis in humans was traced to contaminated injectable glucocorticoids compounded by a pharmacy. This incident, which led to 64 deaths and over 750 illness, redirected Congress towards effort to increase FDA’s ability to regulate compounding. As such, the law was intended to correct what the 1996 FADMA was not able to accomplish. High points of the bill, which was passed in 2013, include: exemption of compounded drugs from new drug labeling and track and trace requirements if the drug is compounded by or under the direct supervision of a registered pharmacist and if the compounding takes place in a registered outsourcing facility. Some of the differences between an outsourcing facility and a traditional compounder include the following. Traditional compounders are either pharmacists or physicians who receive orders for individuals and anticipatory compounding is limited to individuals patients or the physician. In contrast, outsourcing facilities are considered compounding manufacturers that can compound sterile drugs either with or without a prescription. In regards to the source of active ingredients, traditional compounders can compound from FDA approved drugs, USP monographs or a list of “positive” drugs. In contrast, outsource facilities can compound only from the FDA’s shortage list, or the FDA’s list of compounds from bulk. The bulk compounds must be from a registered facility, and accompanied by valid certificates of analysis. Such compounding must be limited to 5% interstate sales or the state of origin must have a Memorandum of Understanding with the FDA. Neither can compound from from withdrawn or removed drugs, or those from the “difficult to compound” list that are considered unsafe or ineffective. Traditional compounders must follow USP monographs if not compounding from the list. Outsourcers, but not traditional compounders, must follow GMP requirements and are subject to risk-based FDA inspection protocols. The facilities must report to the FDA.

The FDA has deemed that the DQSA does not apply to animals. The Government Accountability Office (GAO) has been given the task of addressing the relevance of the law to veterinary medicine. In response, the AVMA has sponsored a Task Force that will advise Congress.

Other Regulatory Considerations

Other considerations of the bill include, but are not limited to the following: The bill calls for publication of a list of drugs (generated through an advisory committee (considered to be difficult to compound and thus might reasonably lead to an adverse effects (safety or effectiveness). The bill improves communication between the federal government and state boards of pharmacy in regards to compounding pharmacies that have been disciplined. The act removes prohibitions in advertising delineated in FADMA. However, a compounded product will be considered misbranded if the advertising or promotion of a compounded drug is false or
misleading. The bill also prohibits resale of a compounded drug labeled “not for resale,” or the intentional falsification of a prescription for a compounded drug.

Several aspects of compounding are outside the regulatory jurisdiction of the FDA. In its compounding CPG, the FDA notes its intent to defer to state authorities (e.g., State Boards of Pharmacy) regarding day-to-day regulation of compounding and to coordinate regulatory efforts with individual states. The Drug Enforcement Agency (DEA) also plays a role in regulation, if the drug in question falls under the jurisdiction of the DEA. For example, a nationally-recognized, internet-based pharmacy that compounds for both human and veterinary medicine (previously mentioned) has recently had its DEA license revoked. In the Federal Register describing this process, the owner of the admitted that over 80% of its sales were made directly to a physician or veterinarian rather than to an individual patient. Examples cited included individual sales of stanozolol injectable, boldenon undecylenate (both to veterinarians) and diazepam injectable (to a physician). Other sales included testosterone, buprenorphine and phenolpropanalamine. Discrepancies in records, inability to account for all substances, volume of drugs being manufactured, and direct sales to veterinarians and physicians are some of the violations cited by the DEA as justification for revocation of the license. Although the loss of the DEA license should not impact compounding of other products by this pharmacy, several of these violations also are violations of the intent of FDAMA and AMDUCA.

State Regulations

In addition to federal laws (AMDUCA, etc), all actions related to pharmacy, including compounding, are regulated by State Boards of Pharmacy. However, individual states vary in the applicability of these laws to compounding veterinarians. Most, but not all states, recognize a veterinarian’s right to compound. Many states have specific regulations for veterinary compounding; in their absence, human compounding regulations apply. Rarely, State Veterinary Medical Boards regulate veterinary compounding. The regulations of the states are quite variable. Some State Boards of Pharmacies allow activities that are clearly in conflict with AMDUCA (such as allowing compounding of animal drugs from bulk substances by some states versus removal of a veterinarian’s right to compound by others). In light of the changes in both human and animal compounding CPG, many State Boards of Pharmacy are re-examining their rules and regulations regarding compounding. The National Association of Board of Pharmacies (NABP; www.nabp.net) is a non-regulatory organization that attempts to provide standards and conformity for individual State Boards of Pharmacy. Currently, this association is generating standard regulatory guidelines (within a model Practice Act) regarding many aspects of pharmacy practice, including compounding, which might be implemented among the states. Because the NABP has recognized the increase in veterinary drug compounding, it has begun to address problems and concerns of the veterinary profession such as compounding by pharmacists that are unaware of differences in regulatory philosophy, or “rogue” pharmacists that are indifferent to the regulations. The NABP website provides a link to each state Board of Pharmacy, which, in turn, generally provides information regarding the state regulations as well as a venue through which queries or complaints regarding inappropriate pharmacy activity can be made. Veterinarians that dispense or prescribe compounded drugs should become aware of the relevant state laws; the AVMA website may be a venue where recently approved or currently considered bills can be reviewed (http://www.avma.org/issues/drugs/compounding/default.asp). It is noteworthy that since the passage of DQSA, several states have implemented new state laws that are intended to address
some of the issues related to compounding. Among the actions are those that address compounding of office stock. According to the AVMA’s State Legislative and Regulatory Affairs Department, currently, 22 states allow veterinary offices to administered compounded products, but specifically prohibit them from dispensing products compounded by a pharmacy; 5 states allow veterinary offices to administered and dispense compounded products, with selected conditions; 11 states allow administration of compounded products, but do not address office dispensing of pharmacy compounded products, and 3 states that have laws and regulations that address compounding in general but not administering or dispensing by practitioners. Seven states have no laws that address compounding.


Compounded Preparations: Need for Standardization

It is unreasonable to expect the Animal Health Institute or the Center for Veterinary Medicine to be able to keep pace with the ever-increasing demand for approved drug products required to treat the wide diversity of animal species and animal diseases. While approved human drugs fill a large need in veterinary therapeutics, those drugs were obviously approved for use in humans and do not always extrapolate well into therapies for animals. Pharmaceutical compounding is an ancient art, thousands of years old, and has always provided veterinary clinicians with the ability to meet therapeutic needs when approved products are not available. Formulas for compounded preparations can be found in ancient Chinese and Egyptian artifacts, pointing to the early awareness that compounded preparations needed to be of a known identity, quality, purity and strength, and that reproducibility from one patient to the next was critical to ensuring therapeutic success from compounded regimens. In 1820 (recent history compared to Egyptian and Chinese archives) several clinicians met in Philadelphia, PA to establish a compendium of compounding standards for medications prepared in the United States with the stated goal that patients should expect to receive the same quality of compounded medication in New York or Philadelphia as they would in any other city in the United States. To that end, the United States Pharmacopeial Convention (USP) was born, and the first edition of compounded preparation recipes was published and available for use by physicians and pharmacists throughout the United States. Since then, the USP compendium of standards has been published 37 times, and in 1975, USP acquired the National Formulary (NF) which is a compendium of excipients and ingredients standardized for use in drug products and compounds.

USP Standards

In 1906, the Food, Drug and Cosmetic Act was passed, officially recognizing the USP as the official set of standards for strength, quality, and purity for all drugs in the United States, and in 1938 The Act was amended to further require that all drugs marketed in the US meet additional standards for packaging, labeling and nomenclature. Although USP is a private standard-setting institution and not a governmental agency, USP standards have become the enforceable standard for all drug manufacturing and compounding in the US. In addition to being the US national standard-setting organization, since its humble inception in 1820, USP has grown to have a global presence with more than 140 countries adopting USP standards. USP uses a uniquely unbiased system of expert volunteers elected by a Convention of delegates from medically-related industries to establish and revise its standards in 5 year terms or Cycles. During each 5 year Cycle, volunteer members of Expert Committees establish USP standards in three different forms: General Notices, General Chapters, and Monographs. General Notices are the overarching standards for drug production, with General Chapters addressing specific processes and behaviors, and Monographs establishing specific standards and performance expectations for individual products or “articles”. General Chapters are numbered either above
The standards in Monographs trump those in General Chapters which trump those in the General Notices in terms of enforceability. Within the Monographs, there are three distinct types of standards: Product Monographs (for FDA approved finished drug products), Substance Monographs (for active pharmaceutical ingredients or bulk chemicals), and Preparation Monographs (for extemporaneously compounded medications). After being carefully developed by expert committees, USP’s standards go out for public comment for a 90 day period in the Pharmacopeial Forum, following which USP must consider and respond to every single public comment issued in response to a standard. Following response to public comments, USP standards are published in the USP/NF as unofficial standards for 6 more months to allow for implementation, following which time, they become official standards of the United States Pharmacopeial Convention and are federally enforceable if so numbered.

Applicability of USP Standards to Veterinary Compounding

Regulation and oversight of compounding has historically fallen to the States, and prior to 2012 a diverse, idiosyncratic system of state rules and statutes evolved causing significant confusion to prescribers and preparers of compounds. Unfortunately, a compounding tragedy of epidemic proportions occurred in October 2012, killing 64 people and injuring more than 700 others following exposure to a compounded preparation of methylprednisolone contaminated with a pigmented fungus, *Exserohilum rostratum*. In the wake of that outbreak, Congress responded quickly with federally enforceable compounding legislation that clearly assigned responsible oversight for compounding to both FDA and state boards of pharmacy. The Drug Quality and Security Act (DQSA) of 2013 divides compounding into “traditional” compounding (one patient, one prescriber, one compound) to be regulated by state Boards of Pharmacy using USP compounding standards, and “outsourcing” compounding (compounds prepared for use in more than one patient that are prepared prior to receipt of a prescription) to be regulated by FDA under Good Manufacturing Practices. Unfortunately, the DQSA applies only to compounding for humans and explicitly states that the DQSA does not apply to compounds prepared for non-humans. So where does regulation for veterinary compounding fall? While the promulgated regulations for the Animal Medicinal Drug Use Clarification Act (AMDUCA) are identified as the only existing veterinary compounding legislation, they are vague and non-specific when it comes to actual oversight of the practice. The regulations rely on a non-enforceable set of Compliance Policy Guides (CPG 608.400) for clarity, and these CPGs have unfortunately created nothing less than a morass of rulings in the courts when challenged. At the time of writing, jurisdiction for veterinary compounding is largely not at the FDA CVM level, but still falls with the States-- primarily with the state Boards of Pharmacy, but secondarily with state Veterinary Medical Boards. Interestingly, state Boards of Pharmacy have all adopted USP Compounding standards as enforceable in an effort to regulate traditional compounding for humans. It is important to note that USP standards do not define “patients” as only human or “compounders” as only pharmacists. It is very clear that USP standards apply to both human and veterinary compounding, and that state Board of Pharmacy inspectors will be well-versed in enforcing USP compounding standards; however, pharmacy inspectors generally have little idea as to how to apply these standards to compounding for non-humans.

USP General Chapters: USP has no less than fifty-five General Chapters that are related to the standardization of compounded preparations. Of these fifty-five General
Chapters, two, Chapters <795> Non-Sterile Compounding, and <797> Sterile Compounding are currently enforceable for veterinary compounding, and one <800> Hazardous Drugs in Health Care Settings is proposed to be enforceable in 2016 following the public comment period. Veterinarians and compounders preparing non-sterile, sterile, and hazardous compounds for animal patients are well-advised to familiarize themselves with these compounding standards and ensure that their compounding practices are appropriately compliant.

**USP Monographs:** USP publishes monograph standards for three types of drug substances: FDA approved products, active pharmaceutical ingredient drug substances, and compounded preparations.

**Product monographs:** USP product monographs are typically provided to USP by the industry sponsor who manufactures that product. Following the development of a USP product monograph, conventionally manufactured drug products must comply with all standards therein. Compliance with a USP product monograph ensures consistency of quality of all FDA approved drugs and enables prescribers to know exactly what specifications to expect from a monographed product. For example, the USP product monograph for *Atenolol Tablets* states a range of acceptable strength to be from 90% - 110% of the labeled amount of atenolol, and the tablets must also comply with certain USP tests for purity, identity, and quality. While the utility of these monographs for compounding may not be immediately apparent, their value becomes quite clear when considering a compounder using an FDA approved product as a starting ingredient for compounding. If the compounder starts with an approved formulation of atenolol tablets, then the strength is known to be within +/- 10%. However, if an approved product does not have a corresponding USP product monograph, the compounder can only guess that the strength stated in the label is “close”. Alarmingy, of the vast number of FDA-approved veterinary products on the US market, only about 12% of those products have USP product monographs. For example, enrofloxacin has multiple FDA approvals in both pioneer and generic presentations; however, a USP product monograph for enrofloxacin does not exist. When a compounder starts with an approved enrofloxacin dosage form, there is no guarantee that the product is within a stated range of strength, purity, identity or quality.

**Substance Monographs:** USP substance monographs are developed by USP experts and represent a narrow standard of purity, identity, strength and quality. The range of potency (strength) for most USP substance monographs varies by only +/- 1.5% instead of +/- 10% as for product monographs. The value of substance monographs is quite clear when it comes to compounding. Compounders utilizing USP monographed substances know the strength of the starting ingredients within a very narrow range as well as the limits for impurities for the substance. Using the example of enrofloxacin, again, there is a USP substance monograph for *Enrofloxacin, USP* even though no product monograph exists. For this reason, the compounder is faced with a dilemma when trying to
prepare a compounded form of enrofloxacin: should the compounder utilize the FDA approved products for which they do not know the expected range of strength? Or should they utilize Enrofloxacin, USP for which they know the strength is within +/- 1.5% and are assured of a limit for impurities? While current FDA interpretation of statute encourages use of the approved veterinary product for compounding, it does not encourage preparation of a compound of resultant known strength.

**Preparation Monographs:** USP compounded preparation monographs represent the most accurate arrow in the compounder’s quiver. Preparation monographs include all of the best elements of product and substance monographs and in addition provide a precise recipe, quality tests, and package, storage and labeling information. The most important information included in a USP preparation monograph is a stability-indicating assay assured beyond-use-date (BUD) which ensures that the compound retains 90% of its strength over the described storage conditions and time period.

The first USP veterinary compounded preparation monographs were developed by AAVPT members serving as volunteer experts on the USP Veterinary Drugs Committee. In the 2000-2005 Cycle, that Committee developed 5 veterinary-specific compounded preparation monographs. During the 2010-2015 Cycle, more than 16 veterinary-specific compounded preparation monographs have become official and many more are in the pipeline for official publication in the next Cycle.

**Summary**

The value of USP compounding standards for treating animal patients cannot be overstated. With the current dearth of compounding regulation and oversight for the production and distribution of compounds for animals, there have been an increasing number of animal casualties as a result of poor quality veterinary compounds. Fortunately, the American Veterinary Medical Association has formulated a Task Force to evaluate core components of meaningful veterinary compounding legislation, but until such statutes are introduced and passed into law by the US Congress, USP compounding standards represent the only viable method for state and federal authorities to ensure the production of quality compounds for animal patients.
Veterinary drug compounding is becoming more and more the norm rather than the exception. Many of the human drugs that we use in veterinary medicine are too concentrated or a dose size inappropriate for our smaller canine and feline patients. Even drugs that are specifically manufactured for use in veterinary medicine may be the wrong dosage size for very small animals or exotic species. Several of the manufactured drugs we commonly use are not available because of raw material shortages or regulatory action against the manufacturers. Just the act of adding a flavoring agent to a commercial product to make it more convenient for owner compliance is considered compounding. Whether we like it or not, compounding is becoming a necessity for treating our patients.

There are many regulatory agencies that monitor compounding by veterinarians and pharmacists: FDA, AVMA, local state legislatures and state boards for both professions. These agencies establish laws mandating which drugs can or cannot be compounded, what quality of material is needed (FDA approved, USP grade) how much can be compounded and guidelines for BUD (beyond use date). Compounding of a drug into a liquid form can be accomplished with two vehicles, aqueous (water based) and oils. The aqueous solutions can only be prepared for a 14 day period unless documentation of stability beyond that has been established. Products compounded in oil can be dispensed with a 180 day expiration date. Solid forms can be compounded into capsules, tablets or flavor tablets and can have an expiration date of 6 months or one quarter of the original drugs expiration date, whichever is shorter.

Some examples of FDA terminology for veterinary compounding are:

FDA- will take action if they detect “compounding” that is clearly outside the bounds of traditional pharmacy practice and violates the act.

FDA- does not distinguish between compounding and manufacturing for drugs used in animals.

FDA- does recognize that unique dosing forms may be necessary for an animal.

FDA- for animals intended for human consumption the veterinarian must establish an extended withdrawal period and that no compounding will result in a residue that may present a risk to public health.
A veterinarian can legally compound medications for their patients once the Veterinary Client Patient Relationship (VCPR) is established. Compounding requires time, equipment and knowledge to complete. The time that is required to accomplish this in your clinic will take you away from what you are truly trained for and want to do, diagnosing and treating animals. Compounding pharmacists are trained for this and compounding is an important part of their practices. Compounding pharmacies will take the prescription order, compound the drug and mail and bill the owner so the burden of the price of the compound is on the pharmacy, not on the veterinarian.

One of the main issues in having a compound prepared for your client is knowing whether the compounding pharmacy is reputable and can correctly and safely compound the drug you are prescribing. There are several guidelines you can use to accomplish this. Look for a pharmacy that is Pharmacy Compounding Accreditation Board (PCAB) accredited or is accredited by another organization. These pharmacies have quality standards required to maintain their certification. This is not to say that a smaller pharmacy that does not have this accreditation cannot safely fill your tablet, capsule and suspension prescriptions correctly, but they may lack the appropriate equipment for sterile compounds. It is a good idea when choosing a local pharmacy to visit with the pharmacists and ask them if they have any experience with veterinary compounding and what veterinary references they use.

A compound may be as easy and simple as adding injectable drugs like detomidine and butorphanol together to give as a single injection, but most are more involved. An example of a time consuming compound is dividing itraconazole or diltiazem capsules, which come with many tiny beads per capsule that need to be divided into smaller doses. This can only be accomplished by placing the beads in smaller capsules and actually weighing each capsule to make sure the finished capsule dose is correct. Liquids are usually simpler. You can determine the mg/ml needed to make a convenient dose for the animal, weigh the powder or measure the liquid and mix the powder or liquid with a suspending agent and a flavoring. Transdermal preparations are more difficult and require a concentrated form of the drug, not just the contents for a capsule or tablet, and use lipoderm gel as the vehicle. Capsules can also be more difficult. You have to determine a capsule size and dilute the powder with a suitable excipient that will fill the capsule to the calculated dose. Then the powder must be packed in the capsule, which is easier with a capsule machine. Some otic and ophthalmic preparations are easily compounded in your clinic and don’t require special equipment or a great deal of your time.

Equipment needed for compounding can be expensive. For example a capsule machine may cost as much as $8,000. Tablet machines are available for about $3,000 and may not be cost effective for a veterinary clinic. Compounding sterile preparations requires a USP (797) compliant isolation glove box or clean room, again impractical for the average clinic. Chemotherapy agents should be compounded in a negative pressure isolation glove box using one of the closed system devices, like PhaSeal, for mixing as well as administration. Drugs that need this type of handling can be supplied by a reputable, accredited compounding pharmacy.
There is now a new class of compounding pharmacies called “outsourcing facilities” under the Drug Quality and Securities Act (DQSA) section 503B. These pharmacies have to register with the FDA and are subject to FDA inspection. They can compound sterile compounds and sell to veterinarians without a specific prescription order required for most compounds. Their products have to undergo sterility and stability testing and documentation much like manufacturers, plus unannounced FDA inspections. There must also be a procedure in place for the compounding to monitor any adverse reactions to their product. The license fee for this type of compounding is $15,000 annually. These outlets are the only facilities that will be allowed to compound sterile products that are currently not available because of raw material shortage or manufacturer regulatory issues. Only a small number of the compounding pharmacies that prepare sterile compounds are signing up for this classification because of the license fee, a portion of which would have to be passed on to the client. Another facet of this act in section 503(a) is the terminology “demonstrable difficulties for compounding”. This is a list of drugs that according to the FDA “could place patients at risk because the final preparation may lead to adverse safety or effectiveness”. This list will apply to all compounders, but according to the FDA does not currently affect compounding for animals.

Veterinary compounding will continue to increase as new drugs are developed that clinicians want to use to treat veterinary patients. Also, as some drugs are dropped from the human field, such as cisapride, diethylstilbestrol and pergolide, they will have to be compounded. Regulations continue to be developed to attempt to assure a quality product for your patients. It is important that you keep current on these regulations and establish a relationship with an accredited compounding.

Additional information on veterinary compounding can be found at:

AVMA Policy on Compounding
www.avma.org/KB/Policies/Pages/Compounding.aspx

Compliance Poly Guide
www.fda.gov/ICECI/CompliancePolicyGuidanceManual/ucm1117042.htm

Pharmacy Compounding Accreditation Board
www.pcab.org
QUESTIONS TO ASK YOUR COMPOUNDING PHARMACIST

Jane G. Owens
Elanco

Veterinarians are faced with many challenges as they seek to provide the best treatment options for their patients. Even though there has been an increase in the number of FDA approved drugs for many of the major species and indications, there remain few approved drugs for minor species and significant gaps in many important indications for major species. Although veterinarians can employ extra-label usage of veterinary and human pharmaceuticals to fill many of these therapeutic gaps, there are times when the approved drug product’s form and presentation are impractical, or in some cases impossible to apply to the patient in need. Recently, many commonly used approved products have become difficult to obtain due to manufacturing or supply issues leading lengthy back orders or unfortunately, complete removal from market. In addition, the prices of some generic drugs commonly used in veterinary medicine, have spiked dramatically.(1) These and other market factors are driving veterinarians to seek alternative ways of obtaining therapies for their patients. However, most veterinarians are not highly trained in chemistry or pharmaceutical sciences and may not be fully aware of how compounded medicines are prepared.

The following are key questions veterinarians should ask their compounding pharmacy to ensure that the compounding pharmacy is providing high quality products that are in compliance with current state and federal regulations in addition to ensuring that they are both safe and effective for their patients.(2)

- Is the compounding pharmacy accredited by an industry organization that sets minimum standards for quality such as the Pharmacy Compounding Accreditation Board (PCAB) or the Accreditation Commission for Health Care (ACHC)? Many veterinary organizations are now requiring that compounding pharmacies be accredited to exhibit at their annual meetings.
- Is the pharmacist trained in animal health dosage forms? The range of dosage forms used in animal compounding is more diverse than those used in human health and importantly, there are significant species differences in the toxicity profiles of excipients used in compounded products. For instance, a common sweetener in compounding, xylitol is 100 times more toxic to dogs than chocolate.
- Are they compliant with United States Pharmacopeia (USP) standards which define good compounding practices? These guidelines are defined in USP chapters 795 and 797.(3,4)
- Are they licensed in the state where the vet practices? Most state boards of pharmacy require that an out-of-state pharmacy register with their state prior to filling prescriptions.
- What source of drug is used in the compounded preparation? FDA approved products are the only legal source for preparing compounded veterinary drugs. Bulk drug substances are the raw active pharmaceutical ingredients that are used to make
final drug products. These bulk drugs are often chemical grade rather than higher quality pharmaceutical grade and do not meet FDA standards for purity, potency and stability. Further the finished compounded products made from these bulk drugs may not have been tested for efficacy or safety in horses or for stability. Often these bulk chemicals are illegally imported from China or India.

- How is this product different than the FDA approved form? If there are no major differences in the formulation as compared to the approved form, the product is essentially an illegally manufactured mimic or ‘pirated’ form. In these cases, the veterinarian should use the legal FDA approved form.

- If the product is in a different form than the FDA approved drug, what evidence shows the finished compounded form will clinically perform as well as the approved product? If no evidence can be presented the veterinarian should be very skeptical that the compounded drug will have equivalent safety and efficacy to the approved product.

- What is the price of the compounded preparation? If the price of the compounded form is significantly less than the cost of the approved version of the same drug, it is likely that the pharmacy is using illegal bulk drug substances in the compounded preparation.

- How was the product tested for potency, purity, stability and sterility (if it is an injectable form)? The veterinarian should ask to see the results from the lot or batch used to prepare the product.

- What is the Beyond Use Date? How was it established and is each lot tested? Is the testing on the bulk ingredients or the finished product? FDA approved products are labeled with an expiry date which is determined by rigorous, long term stability testing. USP describes methods for setting Beyond Use Dates for compounded preparations and they should not be longer than 6 months.(5)

- Are the preparations labeled as ‘non FDA approved”? All compounded preparations are unapproved drugs according to the FDA and the veterinarian is at increased liability risk when using unapproved drugs.

- Is there an adverse event reporting mechanism? What is the pharmacy’s procedure for recall if there is a problem? Veterinarians should report suspected adverse events involving compounded preparations to the compounding pharmacist, the State Board of Pharmacy and the FDA Center for Veterinary Medicine.

- Do they sell compounded products directly to owners? This should be considered a red flag as compounded preparations are by definition extra-label drugs and require a VCPR. Compounded products should only be formulated based upon a valid prescription from a licensed veterinarian within a VCPR.

- Does the compounding pharmacy carry liability insurance? Given recent news reports of owners suing compounding pharmacies, this may be an important consideration.

Certainly there are times when compounded products are necessary to provide for the health and welfare of an equine patient. When that time comes, use a reputable compounding pharmacy and one that can provide answers to your questions. Veterinarians should understand that we are obligated to use an FDA approved product if it is available in the appropriate dosage form and with the relevant label indication in preference to a compounded formulation. Further, when compounding is necessary to tailor the dose and form to our patients, under AMDUCA the
starting point should be an FDA approved product, if available. He or she may also assume increased liability when using compounded products. Compounding a product very similar or identical to an available FDA approved drug product from bulk drug substances constitutes drug piracy and is illegal drug manufacturing.(2)

1) https://www.avma.org/News/JAVMANews/Pages/150115a.aspx
2) JG Owens, Equine Veterinary Education, Scope of Practice: Questions to ask your compounding pharmacist, Sept 2014, IV-V
4) http://www.doh.wa.gov/Portals/1/Documents/2300/USP797GC.pdf
CLINICAL CONSEQUENCES OF DRUG COMPOUNDING

Patricia M. Dowling
University of Saskatchewan

INTRODUCTION

Compounding of veterinary drugs is both necessary and beneficial for the treatment of animals. In October 1994, the Animal Drug Use Clarification Act of 1994 (S 340) was passed as a federal law to codify extralabel drug use (ELDU) in animals by veterinarians. AMDUCA amended the Federal Food, Drug, and Cosmetic Act to legalize ELDU under a valid veterinary-client–patient relationship (VCPR) and other specific conditions according to Food and Drug Administration (FDA) regulations, creating the professional flexibility that veterinarians need to adequately treat animals. Under AMDUCA, ELDU is limited to drug treatment when the health of the animal(s) is threatened or suffering or death may result from failure to treat. Therefore, ELDU should be the exception and not the rule in prescribing and AMDUCA does not encourage ELDU as a way to circumvent the drug approval process. AMDUCA requires that only FDA approved human or veterinary drugs be used can be used in an extralabel manner. The use of compounded drugs is permitted under AMDUCA only when there is not a suitable approved product and compounded drug products can be made only from approved human or animal drug products. Compounded drugs are not the same as generic drugs. Generic drugs are FDA-approved and have demonstrated bioequivalence to the "pioneer brand name" drug product. An approved drug product (brand name or generic) can be identified by the NADA (new animal drug approval number) on its label or by cross-checking with the FDA Green Book of Approved Animal Drug Products. For specific information on compounding of veterinary drugs, see the FDA’s policy guidelines in Sec. 608.400 - Compounding of Drugs for Use in Animals. Unlike regulations that establish “laws”, guidance documents are not binding on the FDA and enforcement is therefore exercised at the FDA’s discretion.

In Canada, approved drugs can be identified by a “Drug Identification Number”. Unlike the United States, where pharmaceutical compounding is federally codified, veterinary compounding in Canada is guided by Health Canada policy, provincial veterinary and pharmacy regulations, and guidelines from the National Association of Pharmacy Regulatory Authorities (NAPRA) and the Canadian Veterinary Medical Association (CVMA).

COMPOUNDING DONE RIGHT

The first step in valid veterinary compounding is the establishment of a valid veterinarian-client-patient relationship. The veterinarian must establish that there is no approved animal or human drug that, when used on label or extralabel according to AMDUCA, can be used to treat the diagnosed condition and that the health of the patient is threatened, or suffering or death may result from failure to treat with the compounded product. The compounding must be performed by a licensed pharmacist upon the prescription of a veterinarian or by a veterinarian if allowed by their state’s pharmacy law. The compounded product must be safe and effective and the compounding operation must be consistent with providing small quantities of product for very specific patient needs.
Preparations can only be compounded from FDA-approved animal or human drugs. If an approved veterinary drug can be used for the compounding, it is not permissible to compound from an approved human drug. The FDA regulations do not permit compounding from bulk (active pharmaceutical ingredient, API) chemicals. Veterinarians may occasionally face situations where they need to treat conditions for which there are no FDA-approved products is available for compounding (e.g., pergolide for horses before Prascend® was approved, cisapride for cats). Although the FDA considers compounding from APIs a violation of the Federal Food, Drug and Cosmetic Act, it acknowledges the need for such compounding within certain areas of veterinary practice and exercises enforcement discretion in such situations.

COMPOUNDING DONE WRONG
When drugs are compounded without adherence to current good compounding practices, veterinary patients may be harmed, the public health may be endangered and the prescribing veterinarian may be liable. Because compounded drugs are typically used in small numbers of patients, if things go wrong it usually does not draw much attention. But recent compounding mishaps were significant enough to draw international attention. In 2009, twenty-one Venezuelan polo horses died acutely in Florida after Franck’s Pharmacy, a veterinary compounding pharmacy, incorrectly prepared a vitamin-and-mineral cocktail that was injected into the horses prior to their polo match. Due to a mathematical error (the easily misplaced decimal point) in the concentration of selenium, the solution was too potent and caused fatal selenium toxicity. In October 2012, an outbreak of fungal meningitis, paraspinal/spinal infections and peripheral joint infections occurred in the United States. The US Centers for Disease Control and Prevention traced the outbreak to contamination with a black mold called Exserohilum rostratum in three lots of preservative-free methylprednisolone acetate solution used for steroid injections. The FDA-approved methylprednisolone acetate (Depo-Medrol®) was sold by Pfizer and two generics companies, but since the compounder’s version did not contain preservatives, it sidestepped the regulatory process with tragic results. Doses from these three lots had been distributed to 75 medical facilities in 23 states, and administered to approximately 14,000 patients. As of May, 2013, 741 people have had infections from the contaminated product and 51 people have died.

Compounding Versus Manufacturing
Unless conditions set forth in 21 CFR 530.13(b) are met, the compounding of a new animal drug from an approved human or animal drug results in an adulterated new animal drug. Traditional compounding was limited to a pharmacist or a physician meeting the therapeutic needs of a specific patient. Once disconnected from individual patients, compounding essentially becomes drug manufacturing. Often compounded products are marketed as cheaper alternatives to the approved drug. All compounding guidelines state that cost is not a defensible reason for prescribing a compounded drug. But in actuality, price is the main reason for using compounded drug products when approved products are readily available. The FDA has taken action against some pharmacies for large scale compounding from bulk APIs and aggressive marketing of such products.¹

¹ According to Health Canada, distinguishing between compounding and manufacturing activities is made on a case-by-case basis. A pharmacy may prepare drugs in very limited
quantities (emphasis Health Canada), in anticipation of a prescription. Yet I have examples of a
compounded meloxicam product that was packaged in a specifically designed premade
cardboard container that would have been made in bulk. The regulation of compounding that
crosses the line into manufacturing is apparently difficult, as evidenced by the widespread sale of
this meloxicam product in this packaging.

Problematic Packaging

Guidelines state that a compounded product should not be made to look like an approved
product. Yet I frequently see packaging that looks very much like an approved veterinary
product and “compounded” is often not indicated on the box or the bottle. In Canada, an
approved human or veterinary drug product has a DIN (drug identification number). One
compounding pharmacy puts a “CIN” number on their products, but it has no meaning other than
to the compounder. I suppose it means “Canadian Identification Number”, but to me it means it’s
a sin to sell this stuff!

For the compounded meloxicam product I referred to earlier, the packaging has a symbol
of a cat on the box. I obtained the product in 2007 before any oral meloxicam formulation was
approved for use in cats in Canada. As there is no package insert of drug information and the
compounder does not provide dosing instructions, a client would easily assume that this product
is intended for use in cats as well as dogs.

Problematic Product

Compounding is legitimate if an approved product is available but the appropriate
method for dosing or the drug concentration is not suitable and a practical alternative does not
exist. The interpretation of a “suitable” product is what frequently leads to the use of a
compounded veterinary product. Health Canada says that the compounded product must provide
a customized therapeutic solution to improve patient care without duplicating an approved drug
product. One of the most frequent examples that I see violating this requirement is compounded
oral liquid phenylbutazone. Considering that there are approved tablet, paste and flavoured
granule formations, it is very difficult to make the case that a liquid formulation is necessary to
save equine lives!

The approved dosage recommendation for meloxicam in dogs is an initial dose of 0.2
mg/kg followed by 0.1 mg/kg daily for maintenance. The original approved meloxicam product
has a concentration of 1.5 mg/ml and is packaged in a bottle that allows dosing by weight with a
specifically designed syringe or by administering by the drop dispenser tip drops that contain
0.05 mg of meloxicam. The compounded product I refer to has a concentration of 3 mg/ml and
does not have a drop dispenser. The enclosed oral syringe fits into a hole in the top of the bottle
and the smallest volume that could be measured accurately would be 0.5 ml – the maintenance
dose for a 15 kg dog. The then suggested and now approved dose of meloxicam for cats is 0.05
mg/kg – a dosage that would be impossible to deliver with this compounded product’s bottle and
syringe for anything less than a 30 kg cat! The potential for overdosing when using this
compounded meloxicam product in dogs weighing less than 15 kg or cats makes it difficult to
justify this more concentrated product as a necessary therapeutic solution to a problem with the
approved products.

There are numerous publications documenting the poor quality of compounded drug
products. 2,3 Many products contain less than the stated drug concentration, but some have been to
contain more drug than stated. 4,5 So there is a much greater chance of under dosing (leading to
therapeutic failure) or overdosing (leading to toxicity) than with an approved drug product. Recently in the US, a compounded pyrimethamine and toltrazuril product for the treatment of Equine Protozoal Myeloencephalitis caused serious adverse reactions and four fatalities in treated horses due to higher than labeled concentrations of pyrimethamine.

In performance horses, there is the additional issue of poor quality compounded drugs resulting in “positive” tests during competition, resulting in loss of purse or prize money, suspensions from competition and the public humiliation of being identified as violating competition drug rules. Recently in Canada, a compounded oral flunixin product was found to contain an additional nonsteroidal anti-inflammatory drug, niflumic acid. Niflumic acid is not available for veterinary or human use in North America and it differs only slightly in structure from flunixin. It appears that imported active pharmaceutical ingredient was the source of the contamination and use of the product lead to drug violations of racehorses and show horses.

Due to the uncertainty regarding residue depletion, compounded drugs should simply not be used in food producing animals. The Canadian gFARAD refuses to give withdrawal recommendations for compounded products. Since the passage of AMDUCA, the FDA has been very clear that compounding of nonapproved drugs from bulk “active pharmaceutical ingredients” in food animals will not be tolerated without specific written approval from the FDA. The only exceptions to this rule are antidotes for use in food animals that are not available as approved products. The FDA will use regulatory discretion to permit compounded formulations of ammonium molybdate, ammonium tetrathiomolybdate, ferric ferrocyanide, methylene blue, pilocarpine, picrotoxin, sodium nitrite, sodium thiosulfate, and tannic acid to be used as antidotes.

**Problematic Prescription Labeling**

State and provincial pharmacy regulations are explicit regarding the information required on a veterinary prescription. I’ve seen numerous instances of mislabelling on compounded products and sometimes they have had no prescription labelling at all. The expiration date of an approved product should be based on known stability data, but stability testing is rarely done by compounding pharmacies for their products. If no stability data exists, products compounded for re-dispensing should have a “beyond use date” identical to the last date the treatment will be administered, as per the duration of the prescription, with a maximum dating of 180 days. A number of publications have demonstrated problems with stability of compounded veterinary drug products such as pergolide and doxycycline.

**PRESCRIBER BEWARE**

If you choose to prescribe compounded drugs, you need to counsel your client regarding potential adverse reactions, including therapeutic failure, and the potential for unintended human or animal exposure to the drug. You are obligated to inform your client that the compounded preparation has not been evaluated by the FDA (or Health Canada) for potency, purity, stability, efficacy or safety, and client consent should be obtained in writing.

As a practicing veterinarian, if you choose to prescribe or dispense a compounded drug, you are responsible for its quality, safety, efficacy and potency and for following pharmacy regulations for proper labeling. You should report suspected adverse events including therapeutic failure and quality defects involving compounded drugs to the compounding pharmacist, the state or provincial Board of Pharmacy and the FDA Center for Veterinary Medicine or Health Canada’s Veterinary Drug Directorate. Should an adverse drug reaction, toxicity or lack of
therapeutic effect occur from a poorly prepared or labeled compounded product, you are responsible for the consequences, which can include client complaints and disciplinary action by your state or provincial veterinary medical regulators. Pharmacies are not required to carry product liability insurance, so you should be sure that your liability insurance will cover you. This may be difficult as there are no “registered label indications” on which an insurance company can base their decision on whether or not a product was used in accordance with best practices. If the manufacture of the compounded product violates federal regulations (e.g. compounded from bulk APIs), your liability insurance will not protect you. So maybe that approved drug product isn’t so expensive after all!

COMPOUNDING GUIDELINES
American Veterinary Medical Association Compounding Policy (https://www.avma.org/KB/Policies/Pages/Compounding.aspx)
FDA Green Book of Approved Animal Drug Products (www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042847)
USP Compounding Standards & Resources (http://www.usp.org/usp-healthcare-professionals/compounding)
CVMA Guidelines for the Legitimate Use of Compound ed Drugs in Veterinary Practice (http://www.canadianveterinarians.net/programs/national-issues.aspx#.UaeFTdj8_kc)
NAPRA Guidelines to Pharmacy Compounding (http://napra.ca/pages/Practice_Resources/guidelines_to_pharmacy_compounding.aspx)

REFERENCES
ABSTRACTS
Pharmacokinetic comparison of oral tablet and suspension formulations of grapiprant, a novel therapeutic for the pain and inflammation of osteoarthritis in dogs

Rausch-Derra L¹, Rhodes L¹, Freshwater L.²

¹ Aratana Therapeutics, Inc., Kansas City, KS, 66103
² BioSTAT Consultants, Inc., Portage, MI 49024

Grapiprant was recently shown to have minimal side effects following chronic (9-month) daily oral administration of 6 mg/kg or 50 mg/kg suspension.¹ The current study compares the pharmacokinetics of this formulation to that of the tablet formulation that will be marketed upon FDA approval. Sixteen Beagle dogs were randomized to receive single doses of either 6 mg/kg or 50 mg/kg grapiprant as both suspension and tablet formulations within a cross-over design with a 15-day washout. Clinical observations were vomiting in one high-dose suspension dog, and loose stools in two dogs, one in each 6 mg/kg formulation group. For both formulations, grapiprant reached a maximum concentration within a few hours. In general, the tablet formulation had better bioavailability, with mean AUCₜₐₐₜ values 34% higher at 6 mg/kg and 64% higher at 50 mg/kg compared to the suspension. Results on Day 0 were similar to those reported on Day 15, suggesting little to no accumulation. Using conversion factors of 1.34 and 1.64, these findings suggest that the 6 mg/kg and 50 mg/kg suspension dosages that were used in the safety study are equivalent to 4.5 mg/kg and 30.5 mg/kg tableted dosages, respectively. Combining these findings with the 9-month safety study demonstrates that safety was evaluated at doses approximately 15-fold above the average demonstrated therapeutic dosage of 2 mg/kg and 10-fold over the ‘safety dose’, defined as the maximum dose a dog of any body weight could receive when dosed at 2 mg/kg with whole or half-tablets.

1. Rausch-Derra et al. ACVIM Forum 2014; poster presentation OT-16

Keywords: grapiprant, anti-inflammatory, suspension, tablet, safety
Target Animal Safety of Doxorubicin (DOXOPHOS® VET) Injection in Mongrel Dogs

Hare JE¹; Young KM¹; Couper NP¹; Lobanov N²; Vail DM³

¹Kingfisher International Inc., 165 Mostar Street, Unit 8, Stouffville, ON, Canada L4A 0Y2
Phone (905) 642-3712
²Oasmia Pharmaceutical AB, Vallongatan 1, SE-752 28 Uppsala, Sweden
Phone: 011 46 70 697 89 73
³School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706
Phone: (608)-890-1324

This study evaluated the safety of a proprietary formulation of doxorubicin (DOXOPHOS® VET) when administered intravenously at 25, 30, and 35 mg/m² once every three weeks for five consecutive treatments in Mongrel dogs. Thirty two (32) dogs were randomized to gender-balanced groups and administered either saline or DOXOPHOS® VET (doxorubicin) at 0.83 (25 mg/m²), 1 (30 mg/m²), or 1.17 times (35 mg/m²) the proposed dose. Dogs were subjected to food consumption measurements and clinical and dose site observations. Periodic evaluations were made of body weight, physical examination, clinical pathology, buccal mucosal bleeding time, and echocardiography. At study completion, dogs were necropsied and histopathology was performed on tissues from all dose groups. All dogs completed the study. Known clinical signs of doxorubicin toxicity were evident in DOXOPHOS® VET treated dogs including gastroenteritis (diarrhea ± vomition and bloody and/or malenic feces), progressive alopecia, reduced weight gain, and testicular atrophy. Changes in peripheral blood included severe dose-dependent neutropenia with a nadir seven to nine days post-dosing. Treated dogs also demonstrated moderate dose-dependent lymphopenia, subclinical thrombocytopenia, and mild anemia. Reduced cell counts were generally transient and rebounded within each dosing cycle. Histopathological changes in treated dogs included dose-dependent lymphocyte depletion in spleen, thymus, lymph nodes, and gut associated lymphoid tissues. Dose-dependent subclinical myocardial degeneration, a known effect of cumulative doxorubicin administration, was histologically evident in treated dogs. In general, however, the adverse effects DOXOPHOS® VET were consistent with those of other chemotherapeutic agents and were either self-limiting or were readily manageable with supportive care.

Keywords: doxorubicin, dog, safety, myelosuppression
Determining canine skin concentrations of terbinafine for the treatment of *Malassezia* dermatitis

Jacqueline R. Gimmler-Mercer, DVM*, Amelia G. White, DVM, MS, DACVD*, Robert A. Kennis, DVM, MS, DACVD*, Crisanta Cruz-Espindola, BS†, Dawn M. Boothe, DVM, MS, PhD, DACVIM, DACVCP†

*Department of Clinical Sciences, College of Veterinary Medicine, 1220 Wire Rd, Auburn University, Auburn, AL, USA, 334-844-4690 E-mail (jrg0024@auburn.edu)

†Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, 109 Greene Hall, Auburn University, Auburn, AL, USA, 334-844-4427

Terbinafine, an allylamine antifungal, is known to concentrate and persist in human skin. To determine if the same is true in dogs, terbinafine (Liconsia, Guadalajara, Spain) was given at 30 mg/kg *per os* once daily to ten dogs for 21 days. Samples of serum, stratum corneum, and sebum were collected from the thorax and paw on Days 1, 5, 7, 11, 14, 21, 28, and 35. High-performance liquid chromatography was used to determine drug concentrations. Four *Malassezia pachydermatis* isolates were collected from routinely submitted fungal cultures. Terbinafine minimum inhibitory concentration (MIC) was determined using a broth macrodilution assay. The highest terbinafine MIC of *M. pachydermatis* was 0.008 mcg/ml. Relevant (mean ± standard deviation) parameters for terbinafine in serum, paw stratum corneum, thorax stratum corneum, and sebum, respectively were: $C_{\text{max}}$ (mcg/mL) 23.59±10.41, 0.31±0.26, 0.30±0.32, and 0.48±0.25; half-life (days) 4.49±2.24, 6.34±5.33, 4.64±3.27, and 5.12±3.33; area under the curve (Day 0-35, day*mcg/mL) 296.78±132.68, 2.59±1.50, 2.46±2.28, and 4.71±3.12; and ratio of Day 21 concentration to MIC ($C_{21}$/MIC) 1907.10±1248.40, 14.91±19.88, 12.98±7.03, and 18.51±7.66. Terbinafine was above MIC after discontinuation in 89, 67, 44, and 78% of dogs, respectively (after 7 days) and 67, 33, 33, and 44% (after 14 days). These results suggest that terbinafine does not concentrate highly in canine skin compared to serum and that time above MIC is variable after discontinuation. Nonetheless, concentrations were generally above the terbinafine MIC for *M. pachydermatis* in all samples during dosing, which could explain the efficacy seen when used for the treatment of *Malassezia* dermatitis.

**Keywords:** terbinafine, *Malassezia*, antifungal
The EMA / CVMP Guideline on the demonstration of palatability of veterinary medicinal products and its practical implementation

Hellmann, Klaus (Dipl. ECVPT) and Claudia Schneider (Dr. med. vet.)

KLIFOVET AG, Geyerspergerstr. 27, D-80689 Munich, Germany; Tel. +49 89 5800820; Email klaus.hellmann@klifovet.com

On 1FEB2015, a new guideline came into effect in the EU. Marketing authorisation holders, who wish to claim palatability for their product, will have to provide data supporting such claim. The guideline provides guidance on the design, conduct and evaluation of studies for demonstration of palatability for the treatment of individual animals, both for new oral formulations as well as existing products if reformulated. The guideline also applies for generic products intended for herd/group treatment as this is considered important with regard to efficacy and safety being not covered in the bioequivalence guideline. It is required to demonstrate palatability in VICH-GCP compliant in-vivo studies in each species and potentially subgroups, assuring that it is representative for the target population.

Assessment of palatability should be without food and reflecting the administration as specified in the SPC. The study design should demonstrate the voluntary full consumption within maximum offering time (e.g. 2 minutes), analysed as the acceptance rate. Non-acceptance is defined by one of the following criteria: delayed uptake, partial uptake, regurgitation or spitting out and refusal. Secondary endpoints may be the average voluntary acceptance rate for each time point, scoring of ease of administration and rates of the different failures as defined in the protocol. Any study outcome must be relevant for the target population, which may be affected by different factors (e.g. conditioning, breed, disease, feeding). Generics intended for oral treatment of herds or groups and not being qualitatively and quantitatively comparable to the reference product need specific attention.

References:
1. EMA/CVMP/EWP/206024/2011: Guideline on the demonstration of palatability of veterinary medicinal products
2. EMA/CVMP/016/00-Rev.2: Conduct of bioequivalence studies for veterinary medicinal products

Key Words: Palatability, veterinary medicinal products
The EMA / CVMP Guideline for the conduct of efficacy studies for non-steroidal anti-inflammatory drugs and its practical implementation

Klaus Hellmann (Dipl. ECVPT) and Caroline Zauter

KLIFOVET AG, Geyerspergerstr. 27, D-80689 Munich, Germany; Tel. +49 89 580082 0; Email klaus.hellmann@klifovet.com

With effect of 1AUG2014, the updated guideline came into force in the EU. NSAIDs are characterised as substances that can be defined by clinical-chemical tests \textit{ex vivo} for their ability to inhibit isoforms of the enzyme cyclooxygenase, which catalyses the conversion of arachidonic acid into prostaglandins and thromboxane \textit{in vivo}. The guideline may be extended to studies aimed at demonstrating efficacy of similar anti-inflammatory agents. The scope of the guideline is to provide guidance on trial design and conduct, as well as on reporting standards for efficacy studies submitted to regulatory authorities.

Clinical studies should be planned based on the route of administration, dosage, frequency and duration of administration. General requirements include randomisation, blinding, analytical and statistical methods and GCP or GLP compliance. Pharmacodynamic and –kinetic data on the active substance are considered important and PK/PD can be an aid to establish the dosing strategy. The selection of relevant primary and secondary endpoints is paramount for pivotal efficacy studies, which should be based on clinically relevant objective measurements. However, as objective endpoints are often missing, subjective assessment methods may be acceptable provided that their validity can be justified. Rating scales are a relevant approach, but validated methods are present in few species and indications. The guideline describes prerequisites for rating-methods to be accepted. Primary endpoints should be supported by secondary endpoints and be feasible for laboratory and field conditions and be ethically acceptable in EU. Non-inferiority and superiority study designs may be acceptable, if justified. Species examples will be given.

References:

1. EMA/CVMP/EWP/1061/2001: Guideline for the conduct of efficacy studies for non-steroidal anti-inflammatory drugs

Key Words: NSAID, efficacy studies, endpoint, scores
Co-administration of phenylbutazone with methocarbamol decreases methocarbamol clearance.

Mary A. Robinson VMD, PhD\textsuperscript{1,2}, Carisa Dixon Tate MS\textsuperscript{1}, Dan Taylor BS\textsuperscript{2}, Ray Boston PhD\textsuperscript{1}, Cornelius Uboh PhD\textsuperscript{2}, Lawrence R. Soma VMD, DACVA\textsuperscript{1}

\textsuperscript{1} University of Pennsylvania, School of Veterinary Medicine, Department of Clinical Studies – New Bolton Center 382 West Street Road, Kennett Square, PA 19348, USA Phone: 610-925-6610; Email: marobins@vet.upenn.edu

\textsuperscript{2} PA Equine Toxicology & Research Laboratory 220 East Rosedale Avenue, West Chester, PA 19382, USA

Methocarbamol is a centrally acting skeletal muscle relaxant that is commonly co-administered to horses with the non-steroidal anti-inflammatory, phenylbutazone. Both drugs must be below designated threshold concentrations in post-race blood samples collected from horses competing in the pari-mutuel racing industry. Veterinarians prescribing methocarbamol reported that withdrawal times based on published studies of the intravenous administration of methocarbamol were inadequate. The presence of a drug-drug interaction with phenylbutazone was hypothesized to explain the discrepancy. To test this hypothesis, nine horses were administered two protocols using a randomized cross-over design. Protocol 1 consisted of the administration of 2.2 mg/kg methocarbamol as a single intravenous bolus. Protocol 2 consisted of the administration of 2.2 mg/kg oral phenylbutazone for 5 days followed by the co-administration of 2.2 mg/kg methocarbamol and phenylbutazone (2.2 mg/kg) on day 6 as single intravenous boluses delivered 30 minutes apart. Methocarbamol and phenylbutazone plasma concentrations were measured using validated LC-MS-MS methods. The decline in methocarbamol plasma concentration following either protocol fit a three compartment mathematical model best. Clearance of methocarbamol was significantly decreased when phenylbutazone was co-administered with methocarbamol (408.5 ± 85.7 mL/h/kg versus 301.2 ± 65.3 mL/h/kg). A longer withdrawal time is needed when methocarbamol is co-administered with phenylbutazone.

**Key words:** drug-drug interaction, methocarbamol, phenylbutazone, equine, withdrawal time
Pharmacokinetic Comparison of a Novel Engineered Particle Formulation of Insulin with Exubera Administered to Beagle Dog.

Philip J. Kuehl¹, Alan Cherrington³, Dan E. Dobry², Dale Edgerton³, Dwayne T. Friesen², Charles Hobbs¹, Chet L. Leach¹, Matthew Reed¹, David T. Vodak², David K. Lyon² and Christopher Tudan¹.

¹Lovelace Respiratory Research Institute: 2425 Ridgecrest Drive SE, Albuquerque, New Mexico 87108, USA. Ph: (607) 229-2461 / (505) 348-940. e-mail: ctudan@lrri.org
²Bend Research Inc.: 64550 Research Road, Bend Oregon 97701-8599 USA
³Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0615 USA

Innovative aerosol products have been shown to be effective for respiratory and atopic diseases. Moreover, this therapeutic approach has also been instrumental in providing more effective bioavailability for medications related to other diseases. The current study describes how in vitro and in vivo inhalation performance of a model spray-dried powder of insulin (Exubera®) and dextran 10 was effective when administered to female beagle dogs. While previous studies have shown similar pharmacodynamic profiles for inhaled insulin when compared to subQ delivery, the bioavailability of inhaled insulin is low. Therefore a novel spray dried formulation of insulin with Dextran 10 as an excipient was developed. The novel formulation of 30% Dextran 10 and 70% insulin and Exubera were evaluated for delivery efficiency and particle size. Both formulations showed similar aerosol delivery efficiency (~ 80%) and particle size (~ 3 µm MMAD). A dose of 1.4 mg/kg of insulin was delivered to female beagle dogs in a cross over study. Immediately following the inhalation of insulin, Somatostatin was infused into a cephalic or saphenous vein to inhibit endogenous insulin secretion. At periodic time intervals following exposures, blood samples were obtained and processed to obtain plasma for C-peptide and insulin analysis. Pharmacokinetic modeling was conducted using non compartmental analysis. The blood analysis showed that both formulations had similar T_max (Dextran 10/insulin: 15 min, Exubera: 10 minutes), and C_max (Dextran 10/insulin: 115 µU/mL, Exubera: 114 µU/mL). However, after dose normalization, the Dextran 10/insulin formulation resulted in a 13% higher relative bioavailability (determined from AUC_0-last).

Key Words: Aerosol Delivery, Insulin, Inhaled Insulin, Dog, Pharmacokinetic
Antimicrobial prescribing patterns for treatment of *Escherichia coli* infections in dogs and cats in the United States

Kamoltip Thungrat, DVM, PhD; Dawn M. Boothe, DVM, PhD, DACVIM (Internal Medicine), DACVCP

Departments of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL; Tel: 334-844-6070, 334-844-4571; E-mail address: kzt0006@auburn.edu

The purpose of this study is to describe antimicrobial prescribing patterns for treatment of feline and canine *E. coli* infections and to identify associated factors including demographics, signalment and patient conditions. *E. coli* clinical isolates were collected from presumed canine (n=610) and feline (n=213) infections diagnosed in veterinary practices in the US between January 2008 and January 2013. A questionnaire was sent to submitting veterinarians. Among the information collected was clinic and patient demographic information, infection location, antimicrobial drug and dosing regimens, and infection response. The majority of *E. coli* infections were canine (74%) from the urinary tract (72%). Antimicrobials were used to treat 93% (95% confidence interval, 89.7-94.8%) of animals. Most (74%) antimicrobials were selected empirically, that is before culture results were obtained. The most frequent choices were amoxicillin-clavulanic acid (AMX; 25%), enrofloxacin (22%), cephalaxin (9%), amoxicillin (9%), and ciprofloxacin (8%). Mean oral doses (mg/kg) were AMX (14.9±5.4) q12h, and enrofloxacin (5.6±2.8) q24h. Based on antimicrobial susceptibility test, 6% of enrofloxacin, 24% of AMX, and 99% of cephalaxin were incorrect choices for resistant *E. coli* infections. Ciprofloxacin was the drug most commonly used for multidrug resistant *E. coli* infections (OR=2.2; *P*<0.05). Response rates were 67% AMX and 69% for enrofloxacin. This study indicates that antimicrobial choices are empirical even when culture is collected, that animals respond despite “incorrect” choices 42% of the time, yet approximately 30% failure should be expected and that antimicrobial choice and dosing regimens warrant reconsideration regarding appropriateness.

**Keywords:** antimicrobials, prescribing-behaviors, infections
Predicting Asymptomatic Bacteriuria in Clinical *Escherichia coli* Uropathogens

Kamoltip Thungrat¹, DVM, PhD; Dawn Merton Boothe¹, DVM, PhD, ACVIM (Internal Medicine), ACVCP; D. Mark Carpenter,² PhD

Departments of Anatomy, Physiology and Pharmacology¹, College of Veterinary Medicine, and the Department of Mathematics & Statistics², College of Science and Mathematics, Auburn University, AL; Tel: 334-844-6070, 334-844-4571; E-mail address: kzt0006@auburn.edu

*Escherichia coli* (*E. coli*) is commonly associated with urinary tract infections (UTI). UTI is associated with clinical signs that range from absent (asymptomatic bacteriuria; ABU) to severe. Severity reflects the presence of different virulence factors (VF) which facilitate survival in the urinary bladder but also induce host clinical signs. In humans, ABU is not an indication for antimicrobial therapy. Treatment may increase antimicrobial resistance, and removal allows infection by more pathogenic organisms. This study aimed to predict clinical *E. coli* from canine UTIs associated with differing severities of clinical signs. Principal Components (PC) Analysis was used to explore relatedness of gene expression. *E. coli* (n=68) cultured from dogs with UTIs were classified as to severity of clinical signs: absent (ABU; n=15), mild (n=18), moderate (n=15), and severe (n=20). A second analysis considered ABU vs non-ABU (n=53) which was mild, moderate and severe combined. Relative RNA expression for VF genes (adhesins [*papG, papC, fimH* and *focA*], toxins [*hlyD* and *cnf1*] and siderophores [*ireA*] was determined by quantitative PCR. Levels of 7 VF genes expression were computed to identify the most related PC and Linear discriminant function (LDF) analysis was then used to model the linear relationship among these selected PCs. Initial analyses yielded 7 PCs, the first 4 of which were subjected to LDF. LDF successfully classified severity of UTI into 2 groups, either ABU or non-ABU, with a 9.3% error rate and 0% false negative rate. These data supported our goal of identifying a “no-antimicrobial” option for animals with ABU.

**Keywords:** virulence, urinary tract infection (UTI), dogs
Antimicrobial susceptibility patterns of clinical *Escherichia coli* isolates from dogs and cats in the United States: January 2008 through January 2013

Kamoltip Thungrat¹, DVM, PhD; Dawn Merton Boothe¹, DVM, PhD, ACVIM (Internal Medicine), ACVCP, D. Mark Carpenter,²PhD

Departments of Anatomy, Physiology and Pharmacology¹, College of Veterinary Medicine, and the Department of Mathematics & Statistics², College of Science and Mathematics, Auburn University, AL; Tel: 334-844-6070, 334-844-4571; E-mail address: kzt0006@auburn.edu

*Escherichia coli* is among the most common bacterial pathogens in dogs and cats. The lack of a national monitoring program limits evidence-based empirical antimicrobial choices in the United States. This study describes antimicrobial susceptibility patterns for presumed clinical *E. coli* isolates from dogs (n=2392) or cats (n=780) collected from six geographic regions in the United States between May 2008 and January 2013. Minimum inhibitory concentrations (MIC) were determined for 17 drugs representing 6 drug classes. Urinary tract isolates were most common (71%). Population MIC distributions were generally bimodal with the second mode above the resistant breakpoint for all drugs except gentamicin, amikacin, and meropenem. The MIC₉₀ exceeded the susceptible breakpoint for ampicillin, amoxicillin-clavulanic acid, cephalothin (surrogate drug for cephalexin), and doxycycline but was below the susceptible breakpoint for all others. None of isolates was susceptible or resistant to all drug tested; 46% were resistant to 1 or 2 antimicrobial categories, and 52% to more than three categories. The resistance percentages were as follows: doxycycline (100%), cephalothin (98%) > ampicillin (48%) > amoxicillin-clavulanic acid (40%) > ticarcillin-clavulanic acid (18%) > cefpodoxime (13%), cefotaxime (12%), cefoxitin (11%), cefazolin (11%), enrofloxacin (10%), chloramphenicol (9.6%) > ciprofloxacin (9.2%), ceftazidime (8.7%), trimethoprim-sulfamethoxazole (7.9%), gentamicin (7.9%) > meropenem (1.5%), amikacin (0.7%) (*P< 0.05*). Resistance to ampicillin and amoxicillin-clavulanic acid was greatest in the South-Central region (*P<0.05*). *E. coli* resistance may preclude empirical treatment with doxycycline, cephalexin, ampicillin, or amoxicillin-clavulanic acid.

**Keywords:** antimicrobials, resistance, *E. coli*, infections
Immunoglobulin G pharmacokinetics in calves fed a colostrum replacer.

Tomas Martin-Jimenez\textsuperscript{a}, DVM, PhD, DACVCP, DECVP; Sharif S. Aly\textsuperscript{b,c, BVSc,MPVM, PhD; Patrick Pithua\textsuperscript{d}, BVSc, MSc, PhD; Ángel García Muñoz \textsuperscript{e}, DVM, PhD; Munashe Chigerwe\textsuperscript{f}, BVSc, MPH, PhD; Debbie Haines\textsuperscript{g,h}, DVM, PhD, Deniece Williams\textsuperscript{c}, DVM, MPVM, DACVPM.

\textsuperscript{a} Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, 2407 River Dr., Knoxville, TN, 37996. Phone: +1-865-755-3569. Email: tmartinj@utk.edu
\textsuperscript{b} Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, One Shields Avenue, CA 95616. Phone: +1-559-688-1731. Email: saly@ucdavis.edu.
\textsuperscript{c} Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, 18830 Road 112, Tulare, CA 93274. Phone: +1-559-688-1731. Email: dwilliams@ucdavis.edu
\textsuperscript{d} Department of Veterinary Medicine and Surgery, University of Missouri-Columbia, Columbia, MO 65211, United States. Phone: +1-573-884-1759. Email: pithwap@missouri.edu.
\textsuperscript{e} Departamento de Producción y Sanidad Animal. Facultad de Veterinaria. Universidad CEU Cardenal Herrera. Moncada. 46113 Valencia, Spain. Phone:+34-96-136-90-00. Email: angel@uhc.ceu.es.
\textsuperscript{f} Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, One Shields Avenue, CA 95616. Phone: +1-530-752-8235. Email: mchigerwe@ucdavis.edu.
\textsuperscript{g} Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 5A2. Phone: 306-966-7245. Email: debbie.haines@usask.ca
\textsuperscript{h} The Saskatoon Colostrum Co Ltd, 30 Molaro Pl, Saskatoon, SK, Canada S7K 6A2.

Colostrum replacer (CR) represents an alternative to maternal colostrum in calves. The objective of this study was to evaluate the pharmacokinetics of IgG in newborn calves fed CR.

Five newborn Holstein calves (3 M, 2 F) were separated from their dams immediately after birth and fed 2 doses of a commercial colostrum-replacer (Calf’s Choice Total HiCal, Saskatoon Colostrum Co. Ltd, Canada). Blood samples were collected from the jugular vein before dosing, at 1 h intervals for 36 h, and at 24 h intervals between 36 and 168 h. Serum IgG concentration was determined by radial immunodiffusion\textsuperscript{1}. The serum IgG concentrations were adjusted by the individual baseline IgG values. Non-compartmental pharmacokinetic parameters were estimated individually with Phoenix 64 (Certara, Princeton, NJ). A nonlinear-mixed effects model was fit to the terminal phase data (36-168 h) using Monolix (v. 4.3.3. Lixoft S.A.S., Orsay, France), to preliminarily explore the effect of body weight on the absorption and disposition of IgG. The statistical significance of any relationship was explored using the likelihood ratio test with \(\alpha = 0.01\). The median time to peak IgG serum concentration was 17 hours. The mean peak IgG concentration and half-life were 11.63 g/L (CV=30%) and 10.16 days (CV=11%), respectively. The mean \(V_z/F\) and \(Cl/F\) estimates were 0.64 L/kg (CV=44%) and 0.0018 L/h*kg (CV=43%). Statistically significant relations were observed between body weight and both \(V_z/F\) and \(Cl/F\). The results of this preliminary study suggest that the capacity of calves to absorb colostrum may increase with neonatal body weight.

References:


Keywords: Pharmacokinetics, colostrum replacer, calves, immunoglobulin G.
**Tramadol metabolism to M1 and M2 in dog liver microsomes: Interindividual variability, identification of responsible CYPs, and drug-drug interactions**

Tania Perez DVM MS, Katrina L. Mealey DVM PhD DACVCP, Tamara Grubb DVM PhD DACVAA, Stephen Greene DVM MS DACVAA, and Michael H. Court BVSc PhD DACVAA

*Program in Individualized Medicine, Department of Veterinary Clinical Sciences, 100 Grimes Way, Washington State University College of Veterinary Medicine, Pullman, Washington 99164. Phone: 509-595-1889, email: tperez@vetmed.wsu.edu*

Tramadol is widely used for the treatment of mild to moderate pain. Tramadol is a pro-drug that is metabolized by CYP to active (O-desmethyltramadol, M1, a weak Mu opioid agonist) and inactive metabolites, including N-desmethyltramadol (M2). The overall objective of this study was to quantify interindividual variation in metabolite formation by dog livers and identify possible causes of this variability.

In vitro studies were conducted using tramadol, pooled and individual (n=27) dog liver microsomes (DLMs), recombinant canine CYPs, inducer-treated DLMs, and inhibitors. Formation of M1 and M2 from tramadol was measured by HPLC-MS.

Marked differences between individual (untreated) DLMs were observed for formation of M1 (17-fold) and M2 (40-fold). M1 was formed exclusively by CYP2D15, while M2 was formed primarily by CYP2B11. M1 and M2 formation were selectively decreased by a CYPD15 inhibitor (quinidine) and a CYP2B11 inhibitor (chloramphenicol), respectively. Formation of M2 (but not M1) was greatly increased by phenobarbital (14-fold), while other inducers had minimal effects. Fluconazole was identified as a potent (IC50 < 1 μM) and selective inhibitor of M2 formation, while bupivacaine and maropitant were potent and selective inhibitors of M1 formation.

High variability in M1 and M2 formation between dogs may be associated with differences in expression and activity of CYP2D15 and CYP2B11, respectively. Some drugs commonly coadministered with tramadol (bupivacaine and maropitant) could decrease M1 formation and associated analgesia, while fluconazole could result in increased M1 concentrations and associated analgesia by selective inhibition of M2 formation.

**Key words:** Tramadol, M1, M2, CYPs, inhibition
Measuring the excitation of canine dorsal root ganglia to pruritogens

Joy Rachel Ganchingco, MSc, DVM; Tomoki Fukuyama, DVM, PhD; Wolfgang Bäumer, Dr. Med. Vet. Habil., Dip ECVPT

Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, 1060 William Moore Dr., Raleigh, North Carolina 27607, USA. Tel: 919 513 6501. Email: jrchanga@ncsu.edu

Sensory nerve fibers that innervate skin have their cell bodies in the dorsal root ganglia (DRG), which connects the peripheral nervous system to the central nervous system. Activation of DRG leads to a calcium influx resulting in excitation of the neuron and propagating sensory stimulus. Therefore, measurement of calcium influx is a direct correlation to neuron activation. Currently, there is no published data on the excitation of canine DRG.

The purpose of this study is the development of a canine DRG cell culture for intracellular calcium measurements. DRG from dogs recently euthanized, for reasons unrelated to this study, were collected through a dorsal approach. Single cell suspensions were obtained by mechanical trituration of enzymatically softened ganglia and seeded onto poly-L-lysine- and laminin-coated glass slides. Within 18-24 hours single cell calcium measurements were performed using Fura-2 AM and ratiometric UV imaging. To begin, we used the classical pruritogens, histamine and protease-activated receptor 2 (PAR2) agonists, as stimulations to identify excitable neurons.

Thus far, we have obtained pilot data from four dogs. From 322 excitable neurons 71 (22.0%) responded to histamine (10-100 µmol/l) with a specific calcium influx. Two dogs showed a positive response to the canine PAR2 agonist (SLIGKT-NH₂); 55 out of 101 neurons (54.5%) significantly responded to SLIGKT-NH₂ compared to the scrambled protein control (TKGILS- NH₂). Among the dogs sampled there was a significant variation in responding cells. This method will allow for further development of in vitro studies of agonists or antagonists to canine DRG stimulation.

Key words: intracellular calcium imaging, canine dorsal root ganglia, pruritogens
Depletion of Phenylbutazone from Equine Urine and Tissues

Patricia M. Dowling¹, DVM, MSc, DACVIM (Large Animal), & DACVCP², Ron Johnson, DVM, PhD, DACVCP, Joe O. Boison³, PhD

¹Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, Saskatoon, SK; Tel: 306-966-7359; Email address: trisha.dowling@usask.ca; ²Department of Veterinary Biomedical Sciences, Ontario Veterinary College, Guelph, ON; ³Canadian Food Inspection Agency, Saskatoon, SK.

Validated liquid chromatography-mass spectrophotometry (LC-MS/MS) assays were developed for the detection of PBZ and its metabolite oxyphenbutazone (OXPBZ) in equine serum and urine (Maxxam Analytics) and tissues (Canadian Food Inspection Agency). The limits of quantification (LOQ) for the assays were 1 ng/mL for PBZ and 2 ng/mL for OXPBZ residues in serum and urine and 0.5 ng/g for PBZ and OXPBZ in all equine tissues. Utilizing horses destined for slaughter and a high dose PBZ treatment regimen (8.8 mg/kg/day for 4 days), we conducted a pilot study to determine the sampling time points for a full drug depletion study. Pilot study results indicated that blood concentrations of PBZ and OXPBZ depleted rapidly and did not reflect tissue concentrations. Both PBZ and OXPBZ depleted rapidly from equine muscle, being readily detectable only at the first slaughter time point of 7 days post-PBZ administration. For the full depletion study, 20 horses were dosed and slaughtered in groups of 5. While OXPBZ concentrations exceeded PBZ concentrations in urine and were still detectable 42 days post-PBZ administration, no residues of OXPBZ were detectable in muscle, kidney, or liver from 21 days post-PBZ administration. Our results indicate PBZ should be the regulatory marker compound and liver should be the target tissue for regulatory enforcement. In conclusion, even after a high dose PBZ treatment regimen, PBZ and OXPBZ deplete rapidly from equine muscle and a lifetime ban on its use in horses intended for human food is not justified.

Keywords: phenylbutazone, residues, equine
Pharmacokinetics and pharmacodynamics of gamithromycin in pulmonary epithelial lining fluid in naturally occurring bovine respiratory disease in multi-source commingled feedlot cattle

DeDonder KD¹, Apley MD², Li M³, Gehring R⁴, Harhay DM³, Lubbers BV⁵, White BJ², Capik SF¹, KuKanich B⁶, Riviere JE⁴, Tessman RK⁷

¹Diagnostic Medicine/Pathobiology, Kansas State University College of Veterinary Medicine, Manhattan, KS, United States; Tel: 785-532-4041 E-mail: kdd5257@vet.k-state.edu ²Clinical Sciences, Kansas State University College of Veterinary Medicine, Manhattan, KS, United States; ³USDA ARS US Meat Animal Research Center, Clay Center, NE, United States; ⁴Institute of Computational Comparative Medicine, Kansas State University College of Veterinary Medicine, Manhattan, KS, United States; ⁵Kansas State Veterinary Diagnostic Laboratory, Kansas State University College of Veterinary Medicine, Manhattan, KS, United States; ⁶Anatomy and Physiology, Kansas State University College of Veterinary Medicine, Manhattan, KS, United States; ⁷Pharmaceutical Research and Development, Merial, Duluth, GA, United States.

Objectives of this study were to determine if 1) a correlation exists between individual pharmacokinetic parameters and treatment outcome when feeder cattle were diagnosed with bovine respiratory disease (BRD) and treated with gamithromycin and 2) if there was a stronger correlation between treatment outcome and gamithromycin concentration in plasma or in the pulmonary epithelial lining fluid (PELF) effect compartment. The study design was a prospective, blinded, randomized clinical trial utilizing three groups of 60 steers/bulls randomly allocated sham injection or gamithromycin mass medication.

Gamithromycin susceptibility of M haemolytica (n=284) and P multocida (n=254) were determined using broth microdilution. A two compartment pharmacokinetic model with a compartment for gamithromycin in plasma and PELF was developed using rich datasets from unpublished studies. The sparse data from our study were then fit to this model using nonlinear mixed effects modeling to estimate individual parameter values which were used to simulate full time-concentration profiles for each animal, which were analyzed using non-compartmental methods so that classical PK/PD indices could be calculated for plasma and PELF.

Marginally significant positive correlations were found between treatment success and both plasma (P = 0.06) and PELF (P = 0.08) AUC₀₋₄. An increased gamithromycin mean residence time (MRT) in the PELF was associated with a successful case outcome (P = 0.03). Calculated PK/PD indices are indicative that for both M haemolytica and P multocida a larger drug exposure in terms of concentration, and time of exposure, was favorable to a successful case outcome, although no statistical differences were observed.

Key words: pharmacokinetics, pharmacodynamics, bovine respiratory disease, gamithromycin, antibiotic resistance
Effect of Oclacinib on Itch and Inflammation in a Chronic Mouse Model of Allergic Dermatitis


Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, 1060 William Moore Drive, Raleigh, North Carolina 27607, USA., Tel.: 1+919 513 1885. E-mail addresses: wolfgang_baeumer@ncsu.edu

In this study, we investigated the effect of the new janus kinase-inhibitor oclacinib on itch and inflammation in a chronic mouse model of allergic dermatitis. A chronic mouse model of allergic dermatitis was conducted by repetitive toluene-2,4-diisocyanate (TDI) treatment in female BALB/c mice. Oclacinib was orally (30 and 45 mg kg⁻¹) or topically (0.25 and 0.5%) applied 30 minutes before and 4 hours after TDI challenge. After TDI challenge, scratching bouts and ear swelling were measured. Additionally, to elucidate a possible rebound phenomenon, oclacinib was administered orally BID at 45 mg kg⁻¹ for 7 days with TDI sensitization then treatment of oclacinib was discontinued abruptly. Mice were sacrificed and dorsal root ganglia (DRG) were isolated from each mouse 30 min after last TDI challenge (i.e. 24 hours after last oclacinib treatment) for excitation experiments.

Oral treatment with oclacinib significantly reduced scratching behaviour, but had only little effect on the inflammatory response, whereas topical treatment significantly improved both itch and inflammatory response. In the rebound experiment, mice treated orally with oclacinib showed again a significant decrease in itch throughout the 7 days of treatment. However, after abrupt withdrawal of oclacinib scratching bouts were significantly enhanced compared to vehicle treatment group. After termination of the study, we observed a higher response of dorsal root ganglia neurons to the pruritogen interleukin 31 and to tumor necrosis factor α in the oclacinib treatment group compared to vehicle treatment group, indicating a peripheral sensitization to pruritogens.

Key words: oclacinib, allergic dermatitis, itch, inflammation
Impact of carprofen administration on the stress and nociception response in cauter
dehorned calves.

Matthew L. Stock VMD, DABVP\textsuperscript{1,2}, Laura A. Barth VMD\textsuperscript{3}, Nick K. Van Engen BS\textsuperscript{1}, Suzanne T. Millman PhD\textsuperscript{1,2}, Ronette Gehring BVSc, MMEdVet (Pharm), DACVCP\textsuperscript{4}, Chong Wang PhD\textsuperscript{1}, Larry W. Wulf PhD\textsuperscript{5}, Walter H. Hsu DVM PhD\textsuperscript{2}, Erica A. Voris BS\textsuperscript{5}, Johann (Hans) F. Coetzee BVSc, Cert CHP, PhD, MRCVS, DACVCP, DACAW\textsuperscript{1,5}

\textsuperscript{1} Department of Veterinary Diagnostic and Production Animal Medicine (VDPAM), College of Veterinary Medicine, Iowa State University (MS: mstock@iastate.edu, 515-294-5971; NVE: nkve@iastate.edu, 515-294-3837; SM: smillman@iastate.edu, 515-294-2871; CW: chwang@iastate.edu, 515-294-3836; JC: hcoetzee@iastate.edu, 515-294-7424)
\textsuperscript{2} Department of Biomedical Sciences (BMS), College of Veterinary Medicine, Iowa State University (WH: whsu@iastate.edu, 515-294-6864)
\textsuperscript{3} Department of Veterinary Clinical Sciences (VCS), College of Veterinary Medicine, Iowa State University (LB: lbarth@iastate.edu, 515-294-2060)
\textsuperscript{4} Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, 66506, USA (rgehring@vet.k-state.edu, phone: 785-532-5660)
\textsuperscript{5} Pharmacology Analytical Support Team (PhAST), College of Veterinary Medicine, Iowa State University (LW: spuds@iastate.edu, 515-294-3138; EV: eavoris@iastate.edu, 515-294-3138)

The objective of this study was to investigate the analgesic effects of carprofen administered immediately prior to cauter dehorning. Forty Holstein calves aged 6 to 8 weeks old were either sham dehorned (n=10) or cauter dehorned following administration of carprofen (1.4 mg/kg) subcutaneously (n=10), orally (n=10), or a placebo (n=10) in a randomized controlled trial. All animals received local anesthesia prior to dehorning. Nociception and stress responses including mechanical nociception threshold, ocular temperature, and heart rate were evaluated following cauter dehorning at predetermined times. Blood samples were collected over 96 hours and analyzed for plasma cortisol and substance P concentrations as well as carprofen and ex-vivo prostaglandin E\textsubscript{2} concentrations. Data were analyzed using a linear mixed effects model with repeated measures. Dehorning was associated with increased nociception throughout the study and a stress response immediately after dehorning, following the loss of local anesthesia, and 48 h post-dehorning compared to calves sham dehorned. Both subcutaneous and oral carprofen administration was well absorbed and achieved concentrations that moderately inhibited ex-vivo prostaglandin E\textsubscript{2} concentrations compared with placebo treated calves. Carprofen treated calves tended to have less pain sensitization (P=0.096) and have a decreased maximum cortisol release (P=0.10) following dehorning compared to placebo treated dehorned control animals; however no differences were observed in other response variables among treatment groups of dehorned calves. Given the observed tendency towards reduction of pain and stress associated with dehorning, further dose titration studies of carprofen in calves are warranted.

Key words: Non-steroidal anti-inflammatory drugs, dehorning, welfare, pain, stress
Pharmacokinetics and pharmacodynamics of the oral factor Xa inhibitor apixaban in cats.

Jennifer A. Myers, DVM, MS, DACVIM (Cardiology); Luke A. Wittenburg, DVM, PhD, DACVCP; Christine S. Olver, DVM, PhD, DACVP; Caitlyn M. Martinez, BS; Janice M. Bright BSN, DVM, MS, DACVIM (Cardiology, Small Animal Internal Medicine)

1 Triangle Veterinary Referral Hospital 608 Morreene Road, Durham, NC, 27705, 919-489-0615, jennmyerscanada@gmail.com
2 Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, 300 West Drake Road, Fort Collins, CO, 80523, 970-297-5000, luke.wittenburg@colostate.edu
3 Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine, Colorado State University, 300 West Drake Road, Fort Collins, CO, 80523, 970-297-5000, colver@colostate.edu
4 Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine, Colorado State University, 300 West Drake Road, Fort Collins, CO, 80523, 970-297-5000, caitlynromero@yahoo.com
5 Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, 300 West Drake Road, Fort Collins, CO, 80523, 970-297-5000, Janice.bright@colostate.edu

Thromboembolism is a common complication of cardiac disease in cats. Anticoagulants are a promising strategy to treat and prevent thromboembolism in these patients, but use of this class of medications is currently limited by challenges in monitoring therapeutic drug levels and by cost. Apixaban is a novel factor Xa inhibitor approved to prevent thrombus formation in human patients with atrial fibrillation. This study aimed to investigate the pharmacokinetic (PK) and pharmacodynamic (PD) properties of apixaban in healthy cats.

A single dose of apixaban (0.2 mg/kg) was orally administered to five purpose bred cats, and blood samples were obtained over 24 hours. After a one week washout period, the cats were given 0.2 mg/kg intravenously followed by repeated sampling. Apixaban concentrations in plasma were measured via liquid chromatography tandem mass spectroscopy. PD effects were determined using a commercial factor Xa assay.

Factor Xa activity decreased as a function of time following a single IV or PO dose of apixaban, and good inverse correlation with plasma apixaban concentrations was noted. PK analysis showed that apixaban has a moderate clearance rate, a short half-life and high bioavailability. A two-compartment model fit the IV PK data. Oral PK and PD data were more variable than IV data.

In conclusion, apixaban is an effective inhibitor of factor Xa in cats. Additional studies are necessary to determine multi-dose PK/PD, effect of cardiac disease on PK/PD, dosing recommendations, and efficacy of apixaban in the treatment/prevention of thromboembolic disease in this species.

Key words: Apixaban, anticoagulant, cat
Pharmacokinetics and Pharmacodynamics of 0.01% and 0.001% Itraconazole Baths in Panamanian golden frogs (Atelopus zeteki) for the Treatment of Amphibian Chytridiomycosis (Batrachochytrium dendrobatidis)

Marike Visser DVM¹, Amy Rifkin², Ellen Bronson DVM DACZM³, Dawn M Boothe DVM DACVIM DACVCP MS PhD⁴

¹ 585 Hoerlein Hall, 1500 Wire Rd Auburn AL 36849, 334-844-7187, mzw0004@auburn.edu
² The Maryland Zoo in Baltimore, 1876 Mansion House Drive, Baltimore, MD 21217, 443-552-3389 adrifkin@email.wm.edu
³ The Maryland Zoo in Baltimore, 1876 Mansion House Drive, Baltimore, MD 21217, 443-552-3389 ellen.bronson@marylandzoo.org
⁴ 109 Greene Hall, 1500 Wire Rd, Auburn AL 36849; 334-844-7187; boothdm@auburn.edu

Batrachochytrium dendrobatidis (Bd) has led to a global pandemic in amphibians. Bd infects keratinized stratified squamous epithelium. Death reflects electrolyte depletion secondary to loss of cutaneous epithelial function. The imidazole antifungal itraconazole appears to cure several amphibian species. This study is the first to measure pharmacokinetic parameters of itraconazole at 0.01% and 0.001%. Panamanian golden frogs (Atelopus zeteki) (n=50; total n=100) were exposed to either 0.01 or 0.001% itraconazole solution bath for 10 minutes and sacrificed (n=5) at 0, 30min, 1, 2, 4, 6, 8,12, 24, 36 hours post removal. After rinsing, ventral skin, liver and heart samples were weighed, homogenized in methanol and centrifuged. Itraconazole was quantitated in supernatant using HPLC with UV detection. Non compartmental analysis (sparse data option) was performed on samples above the LLOQ (2ng/ml). Cmax was above the MIC (<1.56 ng/mg) in all tissues at both bath concentrations and was greater in the skin compared to heart and liver. Average Clast at 0.01%: skin 4.8ng/mg, heart 7.3ng/mg, liver 7.8ng/mg; at 0.001% skin 1.7ng/ml, heart 1.9ng/mg, liver 2.5ng/mg This study indicates that itraconazole at 0.001% for 10 minutes will be sufficient to inhibit Bd and confirms that itraconazole is absorbed systemically with this route of administration.

Keywords: itraconazole, frogs, Amphibian Chytridiomycosis, pharmacokinetics, Batrachochytrium dendrobatidis
Pharmacology of the GLP-1 analog exenatide extended-release in healthy cats

AJ Rudinsky,1 CA Adin,1 S Borin-Crivellenti,2 P Rajala-Schultz,3 M J. Hall,1 C Gilor1

1Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, 601 Vernon Tharp St., Columbus, OH 43210. Email: gilor.1@osu.edu

2FAPESP (#2013/00027-6) and FCAV/Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brazil.

3Department of Veterinary Preventative Medicine, College of Veterinary Medicine, The Ohio State University, 1920 Coffey Rd., Columbus, OH 43210.

Exenatide extended-release (ER) is a microencapsulated formulation of the GLP-1 receptor agonist exenatide. It has a protracted pharmacokinetic profile that allows a once-weekly injection with comparable efficacy to insulin with an improved safety profile in type-2 diabetic people.

Here we studied the pharmacology of Exenatide-ER in six healthy cats. A single subcutaneous injection of Exenatide-ER (0.13 mg/kg) was administered on day 0. Exenatide concentrations were measured for 12 weeks. A hyperglycemic clamp (target = 225 mg/dL) was performed on days -7 (Clamp-I) and 21 (Clamp-II) with measurements of insulin and glucagon concentrations. Glucose tolerance was defined as the amount of glucose required to maintain hyperglycemia during the clamp. Continuous glucose monitoring (CGM) was performed on weeks 0, 2 and 6 post-injection.

Plasma concentrations of exenatide peaked at 1 hr and 4 wks post injection. Comparing Clamp-1 to Clamp-2, fasting BG decreased (mean [±SD] = -11 ± 8 mg/dL, P=0.02), glucose tolerance improved (median [range] +33% [4-138%], P=0.04), insulin concentrations increased (+36.5% [-9.9-274.1%], P=0.02) and glucagon concentrations decreased (-4.7% [0-12.1%], P=0.005). Compared to pre-injection values on CGM, glucose concentrations decreased and the frequency of readings <50mg/dL increased at 2 and 6 wks post-injection of exenatide-ER. This did not correspond to clinical hypoglycemia. No other side effects were observed throughout the study.

Exenatide-ER was safe and effective in improving glucose tolerance 3 wks after a single injection. Further evaluation is needed to determine its safety, efficacy and duration of action in diabetic cats.

Keywords: feline, exenatide, incretin, diabetes, insulin, glucagon
Pharmacokinetics and distribution in interstitial and pulmonary epithelial lining fluid of danofloxacin in ruminant and preruminant calves

Danielle A. Lindquist, Marilyn Martinez, Geof W. Smith, Ronald E. Baynes

Department of Population Health and Pathobiology and the Food Animal Residue Avoidance Depletion Program, College of Veterinary Medicine, North Carolina State University, Raleigh 27607; Phone: (919) 636-8063; Email: dalindqu@ncsu.edu

The purpose of this study was to compare the distribution of danofloxacin in pulmonary epithelial lining fluid (PELF) of the lungs in comparison to plasma and interstitial fluid (ISF) concentrations in healthy ruminating (6 month old) vs preruminating (3 week old) Holstein calves. Eight calves in each group were given a single subcutaneous dose (8 mg/kg) of danofloxacin. Plasma, ISF and bronchoalveolar lavage (BAL) fluid were collected over 96 h. Drug assays were performed using high pressure liquid chromatography. ISF samples were collected from ultrafiltration probes. PELF concentrations were calculated from BAL fluid concentrations through quantification of dilution (urea in plasma/urea in BAL fluid) during the BAL procedure. Data were subjected to compartmental pharmacokinetic analysis. On average, there was a trend for slower absorption of danofloxacin in preruminant than in ruminant calves. The time to maximum concentration (Tmax) in the plasma was significantly longer in preruminating calves (3.12 h) versus ruminating calves (1.4 h). Concentrations in ISF tended to be maintained for a longer duration in the preruminant calves. A significant difference was not detected between groups in area under the curve, beta half-life or maximum concentration in plasma. The variability of measured drug concentrations was much greater in preruminant calves than that of ruminant calves in plasma, PELF and ISF. Despite noted differences in absorption rate of ruminant and preruminant calves, the plasma/ELF/ISF results is consistent with the ability to achieve effective drug concentrations when administered as a 8 mg/kg SC dose.

Key words: danofloxacin, preruminant, calves, pharmacokinetics, HPLC
Drug Allergy in Veterinary Medicine: The Survey

Fabrice T. Fosset, DVM, Sidonie N. Lavergne, DVM, PhD

College of Veterinary Medicine, Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL 61802, P: 217-265-0315; E-mail addresses: fabricefosset@gmail.com, slavergn@illinois.edu

Drug allergic reactions are immune-mediated adverse drug events: “immediate” IgE-mediated (≈1/3) or “delayed” IgG/T cell-mediated reactions. This topic is rarely taught in any detail during veterinary training, decreasing clinicians’ awareness and preparedness to diagnose and manage them. We therefore designed a survey through SurveyMonkey that targeted UIUC alumni veterinarians (n=2164) to evaluate their opinion on this matter. We received 275 responses (12.7%) over 8 weeks. 43.8% believed their curriculum had not prepared them adequately to diagnose and treat these reactions; 33.6% indicated that they never received any information about drug allergy during their training; and 50.6% that there isn’t enough information in the veterinary literature. Yet, 78.3% estimated seeing 1-12 confirmed or highly suspicious cases/year, 9.6% 13-24, and 1.7% 25-59. Participants indicated that drug allergy usually involves: skin (97.6%), GI (89.2%), respiratory (88.8%), cardiovascular (77.3%), and blood (69.3%). Initially, most included clinical signs compatible with anaphylaxis only (67.8%), rather than delayed reactions (0.8%) or both (13%). However, after reading a short informative paragraph about drug allergy, 30.8% participants indicated that they would include drug allergy more often in the differential diagnosis and 45.1% said they would start asking about past drug allergy for their patients. 87.7% indicated being interested in learning more about drug allergy as part of their continuing education in the future. In conclusion, this survey suggests that there might be a lack of awareness secondary to a lack of teaching specific to drug allergy in veterinary medicine, like has been identified in human medicine previously.

Keywords: Toxicology, immunology, pharmacology
FEEDBACK
AAVPT 19th Biennial Symposium Feedback Form

How would you rate this symposium? 0= the worst, 10 = the best (please feel free to add comments)

What did you like most about the symposium?

What did you like least about the symposium?

Were the proceedings helpful? What format do you prefer (paper, electronic/website, pen/USB flash drive)?

What would you like to see changed about the symposium? (Consider commenting about the content, order of presentations, length, venue, speaker, organization, etc.)

What topics would you like to see at future Biennial Symposia or AAVPT sponsored workshops?

Please include any other suggestions:
Donation Form

The Veterinary Pharmacology Research Foundation greatly appreciates all financial donations as we work toward our goal of advancing the field of veterinary pharmacology. Donations are tax deductible in the US as the Foundation has been granted tax exempt status under section 501c(3) of the Internal Revenue Code.

The mission of the Veterinary Pharmacology Research Foundation is to provide grant funding to support research to evaluate the safety, effectiveness and duration of effect of therapies for veterinary species, explore new drug therapies for animals, develop and validate methods of evaluating effects of drugs on animal diseases or conditions, ensure that a safe food supply is not compromised by drug therapy and support training programs for veterinary pharmacologists.

Donations may be made by check or money order, made payable to:

The Veterinary Pharmacology Research Foundation

If you would like to make a tax-deductible donation, please print out this form and mail the completed form to:

Dr. Dan Gingerich
Secretary/Treasurer
Veterinary Pharmacology Research Foundation
2219 Wilmington Road
Lebanon OH 45036

Name: _____________________________________________________________
Street Address: ______________________________________________________
City:____________________________ State:___________ Zip:____________
Email Address:_______________________________________________________
Telephone Number:_______________________ Total Amount Enclosed: $________

☐ Please check if you would like your donation to remain anonymous

Thank you for your support of the Veterinary Pharmacology Research Foundation. We are honored that you support this growing foundation which is devoted to advancing the field of veterinary pharmacology.