Predicting Toxicities of Reactive Metabolite—Positive Drug Candidates

AMIT S. KALGUTKAR
MEDICINE DESIGN, PFIZER INC, CAMBRIDGE, MA 02139
AMIT.KALGUTKAR@PFIZER.COM
Safety Related Attrition: Adverse Drug Reactions (ADRs)

- ADRs contribute to patient mortality and morbidity

ADR classification
- Type A ADRs: ~ 80% of all ADRs fall into this category
  - Type A ADRs can be predicted from known primary pharmacology (e.g., hemorrhage with anti-coagulants)
  - Dose dependent; can be reversed with reduction of dose
  - Generally identified in preclinical studies (e.g., animal models of pharmacology, toxicology)
- Type B or Idiosyncratic ADRs (hepatotoxicity, skin rashes, agranulocytosis, etc.)
  - Unrelated to primary pharmacology (e.g., non-tricyclic anti-depressant (CNS agent) nefazodone can cause liver failure)
  - Cannot be predicted from traditional preclinical toxicity studies in animals
  - Can be severe, may be fatal – Most common cause of drug withdrawal or black box warning label
  - Rare occurrence (1 in 10000, 1 in 100000); often observed in phase III or post-launch
Idiosyncratic Adverse Drug Reactions (IADRs) and Chemical Peculiarity (Structural Alerts and Reactive Metabolites)

- Structural alerts are organic functional groups that can generate reactive metabolites (RMs) via the process of bioactivation by drug metabolizing enzymes
  - RMs can be generated by phase I oxidation (e.g., cytochrome P450) or phase II conjugation (e.g., glucuronidation)
    - Common feature of many drugs associated with idiosyncratic drug toxicity

- Downstream consequences of RM formation as it relates to IADRs (particularly the idiosyncratic nature) remains unclear
  - Lack of animal models and clinical biomarkers

Many IADRs are immune mediated

Covalent adduction of low MW reactive metabolites to proteins – immunogen formation
Well-established for penicillin (β-lactam ring opening)-induced anaphylactic reactions
Associating the bioactivation of a structural alert with IADRs

Take the case of poison ivy/oak rash

- The toxic effects of the oxidized urushiols are mediated by an induced immune response.
- The oxidized urushiols acts as haptens, chemically reacting with integral membrane proteins on exposed skin cells (e.g., CD28).
- Affected proteins interfere with the immune system's ability to recognize these cells as normal parts of the body, causing a T-cell-mediated immune response.

Derived from urushi, Japanese name for lacquer

~1 ng of this oily resin needed to cause rash

¼ ounce is all that is needed to cause rash in every human on earth

Associating the bioactivation of a structural alert with IADRs

The sulfonamide motif has achieved notoriety with respect to hypersensitivity (e.g., skin rashes)

BLACK BOX WARNING: FATALITIES ASSOCIATED WITH THE ADMINISTRATION OF SULFONAMIDES, ALTHOUGH RARE, HAVE OCCURRED DUE TO SEVERE REACTIONS, INCLUDING STEVENS-JOHNSON SYNDROME, TOXIC EPIDERMAL NECROLYSIS, FULMINANT HEPATIC NECROSIS, AGRANULOCYTOSIS, APLASTIC ANEMIA, AND OTHER BLOOD DYSCRASIAS. SULFONAMIDES, INCLUDING SULFONAMIDE CONTAINING PRODUCTS SUCH AS TRIMETHOPRIM / SULFAMETHOXAZOLE, SHOULD BE DISCONTINUED AT THE FIRST APPEARANCE OF SKIN RASH OR ANY SIGN OF ADVERSE REACTION.

(Bactrim®)

Reactive Metabolite Liability as a Causative Factor in IADRs

**Drugs Withdrawn**
- Alpidem (anxiolytic), *Hepatitis* (fatal)
- Amodiaquine (antimalarial), *Hepatitis, agranulocytosis*
- Aminophen (antidepressant), *Hepatitis, cutaneous ADRs*
- Benoxaprofen (antiinflammatory), *Hepatitis* (fatal)
- Carbutamide (antidiabetic)
- Bone marrow toxicity
- Ibuprofen (antiinflammatory), *Hepatitis* (fatal)
- Iproniazid (antidepressant), *Hepatitis* (fatal)
- Metiamide (antiulcer)
- Bone marrow toxicity
- Nomifensine (antidepressant), *Hepatitis* (fatal), anaemia
- Practolol (antihypertensive)
- Remoxipride (antipsychotic), *Aplastic anaemia*
- Aplastia (antipsychotic)
- Remoxipride (antipsychotic)
- Aplastia (antiinflammatory)
- Tienilic Acid (diuretic), *Hepatitis* (fatal)
- Tolrestat (antiulcer), *Hepatitis* (fatal)
- Zomepirac (antiinflammatory), *Hepatitis, cutaneous ADRs*

**Marketed Drugs**
- Abacavir (antiretroviral), *Cutaneous ADRs*
- Acetaminophen (analgesic), *Hepatitis* (fatal)
- Captropril (antihypertensive), *Hepatitis, agranulocytosis*
- Carbamezepine (anticonvulsant), *Hepatitis, agranulocytosis*
- Clozapine (antipsychotic), *Agranulocytosis*
- Cyclophosphamide (anticaner), *Agranulocytosis, cutaneous ADRs*
- Dapsone (antibacterial), *Agranulocytosis, cutaneous ADRs, aplastic anaemia*
- Diclofenac (antiinflammatory), *Hepatitis*
- Felbamate (anticonvulsant), *Hepatitis* (fatal), aplastic anaemia (fatal), severe restriction in use, *Furosemide* (diuretic), *Agranulocytosis, cutaneous ADRs, aplastic anaemia*
- Halothane (anesthetic), *Hepatitis*
- Isoniazid (antibacterial), *Hepatitis* (can be fatal)
- Phenytoin (anticonvulsant), *Agranulocytosis, cutaneous ADRs*
- Procarbazine (antiarhythmic), *Hepatitis, agranulocytosis*
- Sulfamethoxazole (antibacterial), *Agranulocytosis, aplastic anaemia*
- Ticlopidine (antithrombotic), *Hepatitis, cutaneous ADRs*
- Tolcapone (antiparkinson), *Hepatitis (fatal)*
- Trazodone (antidepressant), *Hepatitis*

For an exhaustive survey see:
- [Chemical Research in Toxicology](https://pubchem.ncbi.nlm.nih.gov)

### For an exhaustive survey see:

**Structural Alert/Reactive Metabolite Concept as Applied in Medicinal Chemistry to Mitigate the Risk of Idiosyncratic Drug Toxicity: A Perspective Based on the Critical Examination of Trends in the Top 200 Drugs Marketed in the United States**

Antonio J. Sipos,1,2,9 Daniel P. Wallis,1 Joseph R. Runyan,1 David A. Price,1 Thomas A. Ballie,1 Amit S. Kolakovic,2,9 and Michael D. Alder1

---

*1Worldwide Medicinal Chemistry, Pharmacokinetics, Dynamics and Metabolism, and Neuropharmacology, New Brunswick, New Jersey, United States*  
2Department of Medicinal Chemistry/School of Pharmacy, University of Washington, Seattle, Washington, USA.
Examples of Functional Groups (Structural Alerts) Susceptible to RM Formation

- Anilines (masked anilines)
- p-Aminophenols
- Nitrobenzenes
- Hydrazines (phenylhydrazines)
- Benzylamines
- Catechols
- Cyclopropylamines
- 1,2,3,6-Tetrahydopyridines
- 2-Halopyridines and pyrimidines
- Haloalkanes
- Unsubstituted alkenes
- Acetylenes
- Imides
- Formamides
- Sulfonylureas

- Thioureas
- Methylenedioxyphenyl groups
- Reduced aromatic thiols
- 5-Hydroxy(or methoxy) indoles
- 3-Methylindoles
- Unsubstitued furans
- Unsubstitued thiophenes
- Unsubstitued thiazoles
- Unsubstitued oxazoles
- Thiazolidinediones
- Fatty acids (medium to long chain)
- Carboxylic acids
- Hydroxylamines
- Hydroxamic acids
- Michael Acceptors
- Hydroquinones

- Bromobenzene

List is exhaustive; even includes a simple phenyl ring

Impact of RMs in Drug Discovery / Development

- 3 principal areas of concern to be addressed when RMs are formed with lead chemical matter
  - Genotoxicity/carcinogenicity
  - RM covalently modifies DNA
  - Ames test for genotoxicity has S9/NADPH arm to test for RM

> Drug Metab Dispos 2007, 35(6):848-858.

- Mechanism-based inactivation of cytochrome P450
  - RM covalently modifies P450 isoform(s) responsible for its formation
  - Can lead to drug-drug interactions


- Idiosyncratic drug toxicity
  - Covalent modifications of proteins (haptenization)

- Aflatoxin B1 (AFB1) – Fungal mycotoxin/hepatocarcinogen
  - Rate-limiting step is P450-catalyzed RM formation
  - RM (epoxide) reacts with DNA, water and glutathione

- Bergamottin (and 6',7'-dihydroxy derivative) – Components of grapefruit juice
  - P4503A4 inactivation through RM (epoxide)
  - DDI with P4503A4 substrates (e.g., atorvastatin)

- Acetaminophen – Antiinflammatory drug
  - Dose dependent hepatotoxin
  - P450-catalyzed oxidation to RM (quinone-imine)
Current Best Practice in Drug Discovery to Mitigate IADR Risks

• Until a deeper understanding of the downstream consequences of RM formation is gained
  - Exclude the use of structural alerts in drug design
  - Elimination of RM potential of lead chemical matter is a pragmatic approach to mitigate IADRs (structure – toxicity studies are proof)
  - Increasing the pharmacologic potency and/or optimization of the ADME characteristics to achieve low daily dose is an important tactic to avoid attrition due to IADRs
  - Low daily dose are unlikely to be associated with IADRs

**CB-1 Antagonists**  *Arch. Pharm.* 2008, 341, 405-411

- RM + ve (arene oxide) CB to liver microsomes
- No RM No CB Taranabant

**Glucokinase Activators**  *J. Med. Chem.* 2012, 55, 7021-7036

- RM + ve (thiazole ring scission to thiourea) Preclinical hepatotoxicity
- *R,R*-diastereomer (Piragliatin) Completed Phase 2B trials
Drug Withdrawal due to IADRs

Liver toxicity of sitaxsentan in pulmonary arterial hypertension
Eur Respir J 2011; 37: 475–477

Sitaxsentan-induced acute severe hepatitis treated with glucocorticoid therapy
Can Respir J Vol 19 No 1 January/February 2012

Multiple Compound-Related Adverse Properties Contribute to Liver Injury Caused by Endothelin Receptor Antagonists

Bioactivation of Sitaxsentan in Liver Microsomes, Hepatocytes, and Expressed Human P450s with Characterization of the Glutathione Conjugate by Liquid Chromatography Tandem Mass Spectrometry
Chem. Res. Toxicol. 2013, 26:926-936

- Sitaxsentan
- Endothelin receptor antagonist
- Approved for pulmonary arterial hypertension
- Daily dose ~ 100 mg QD

- Marketed in EU and Canada (~ 2008)
- Several cases of liver toxicity (fatal) in phase III trials in USA
- Withdrawn from worldwide market (2010)
  - RMs (and protein covalent binding) demonstrated
  - Inhibitory effects on bile salt export pump (BSEP) also noted in vesicle assays
Recommended Reading

Managing the challenge of chemically reactive metabolites in drug development


Predicting Toxicities of Reactive Metabolite–Positive Drug Candidates

Amit S. Kalgutkar¹ and Deepak Dalvie²

Pharmacokinetics, Dynamics and Metabolism Department, Pfizer Worldwide Research and Development, ¹Cambridge, Massachusetts 02139 and ²San Diego, California 92121; email: amit.kalgutkar@pfizer.com, deepak.dalvie@pfizer.com

Detection of RMs in Preclinical Drug Discovery

**Two Commonly Used Methods**

- **Covalent binding to proteins**
  - Most definitive
  - Useful in quantitation of the reactive metabolite
  - Limited by availability of radioactive compounds
  - Not amenable to HT screening

- **Trapping electrophilic species with nucleophilic reagents**
  - Most popular in a discovery setting
  - Acts as a surrogate marker of covalent binding

Glutathione (GSH)  
*Commonly Used Nucleophile*

Commonly implemented in screens to study RM formation potential of new chemical entities

Metabolism-Toxicity Relationships

• Circumstantial evidence that avoiding RM formation will eliminate toxicity

Enol-carboxamide NSAIDs

Sudoxicam
- Hepatotoxic (acute liver failure)
- Withdrawn from Phase III trials

Meloxicam
- Non-hepatotoxic
- Part of the top 200 most prescribed drugs list

Piroxicam
- Non-hepatotoxic
Differences in Metabolism of Sudoxicam and Meloxicam in Humans

**Sudoxicam:**

- Thiazole ring scission is the principal metabolite route
  - Hepatotoxic potential of thiourea derivatives well established

**Meloxicam:**

- Little to no thiazole ring scission
  - THE STORY OF THE “MAGIC” METHYL GROUP

**Fundamental Message:** Not all structural alert-containing molecules will form RM(s) during the process of metabolism

Illustrations of “Blockbuster” Drugs Containing Structural Alerts that do not Undergo Bioactivation

- **Rivaroxaban (Xarelto)**
  - Oral Anti-coagulant
  - Daily dose = 20 mg
  - Chlorothiophene SA is not bioactivated
  - Metabolic CL involves amide hydrolysis and morpholinone ring oxidation

- **Canagliflozin (Invokana)**
  - Antidiabetic
  - Daily dose = 300 mg
  - Thiophene SA is not bioactivated
  - Metabolic CL involves glucuronidation of the OH groups in the glycoside motif

- **Pramipexole (Mirapex)**
  - Parkinson’s disease
  - Daily dose = 4.5 mg
  - > 90% renal excretion (unchanged)
  - Virtually devoid of metabolic CL
  - Aminothiazole SA is not bioactivated

- **Ranitidine (Zantac)**
  - Anti-ulcer
  - Daily dose = 150 – 300 mg
  - > 90% renal excretion (unchanged)
  - Virtually devoid of metabolic CL
  - Furan SA is not bioactivated
Factors Governing Bioactivation-Dependent Toxicity

- Also important to consider the role of detoxication pathways:
  - Is the RM (or its precursor) readily detoxicated?

- Example: Paroxetine (Paxil) – SSRI for treatment of depression
  - Presence of a methylenedioxyphenyl (1,3-benzdioxole) structural alert
  - Bioactivated by CYP2D6 to carbene intermediate resulting in mechanism-based inactivation of CYP2D6
    - Clinical drug-drug interactions with CYP2D6 substrates well-established
    - Autoinactivation of paroxetine clearance noted in clinical trials

Paroxetine is rarely associated with IADRs (e.g., hepatotoxicity) despite decades of clinical use

Incubation of paroxetine with NADPH- and GSH-supplemented human liver microsomes

- Detection of glutathione conjugates (consistent with bioactivation of the methylenedioxyphenyl group to electrophilic quinone intermediates)
- Covalent binding to human liver microsomes (NADPH-dependent) with $[^{14}\text{C}]$-paroxetine

Highly unlikely that paroxetine would be nominated as a drug candidate in modern drug discovery
Mechanistic Analysis of Paroxetine Bioactivation

Effect of added co-factors and GSH on NADPH-dependent covalent binding of [14C]-paroxetine to human liver microsomes or human liver S9 fractions

Reduction in covalent binding in the presence of added GSH – consistent with trapping of electrophilic quinones

Reduction in covalent binding in the presence of S-adenosylmethionine (SAM)

Q: Why did we add SAM to the human liver S9 incubations?

Impact of SAM on Paroxetine Covalent Binding

Major metabolite of paroxetine in humans is derived from catechol-\(O\)-methylation by catechol-O-methyltransferase enzyme (requires SAM as co-factor)

Paroxetine was bioactivated but:
- \(\alpha\)-Quinone is detoxified by conjugation with GSH
- Catechol detoxified by methylation

Tip: Conjugation of RM to glutathione (GSH) is a detoxication pathway (depletion of GSH can lead to hepatotoxicity (e.g., acetaminophen))
Paroxetine daily dose (~ 30 mg) is lower than acetaminophen (325 -1000 mg)
Level of RM formed with paroxetine could be handled by endogenous GSH concentrations (5 – 7 mM)
Impact of Daily Dose on IADRs

Drugs (n=31) withdrawn due to IADRs

Drugs (n=37) associated with black box warning

Drugs associated with IADRs are frequently the ones with a higher daily dose

> 100 mg (84%)

> 100 mg (81%)
Impact of Daily Dose on IADRs (Particularly DILI)

Relationship Between Daily Dose of Oral Medications and Idiosyncratic Drug-Induced Liver Injury: Search for Signals

HEPATOLOGY. June 2008

Craig Lammert,1 Stefan Einarsson,2 Chandan Saha,3 Anna Niklasson,2 Einar Björnsson,2 and Naga Chalassani4

Idiosyncratic drug-induced liver injury (DILI) is traditionally thought not to be dose-related. However, it has been pointed out that most medicines that were withdrawn from marketing or received a black-box warning because of hepatotoxicity were prescribed at daily doses greater than 50 mg/day. To examine the relationship between daily dose of medications and idiosyncratic DILI, we conducted a study with two aims. First, using two pharmaceutical databases, we examined the relationship between daily dose of commonly prescribed medicines in the United States and reported frequency of their selected hepatic adverse events. Second, we examined serious DILI cases reported to the Swedish Adverse Drug Reactions Advisory Committee (1970-2004) for any signals supporting the relationship between daily dose and idiosyncratic DILI. Medications were categorized into ≤10 mg/day, 11-49 mg/day, and ≥50 mg/day groups. Among US prescription medicines, a statistically significant relationship was observed between daily dose of oral medicines and reports of liver failure (P = 0.009), liver transplantation (P < 0.001), and death caused by DILI (P = 0.004) but not alanine aminotransferase (ALT) > 3 × upper limit of normal (P = 0.10) or jaundice (P = 0.16). Of 598 eligible Swedish DILI cases, 9% belonged to the ≤10 mg/day group, 14.2% to the 11-49 mg/day group, and 77% of cases were caused by medications given at dose ≥50 mg/day. A statistically significant relationship was noted between daily dose and poor outcome (death or liver transplantation) of Swedish DILI cases (7%, 9.4%, and 13.2% in ≤10, 11-49, and ≥50 mg/day groups, respectively, P = 0.03). Conclusion: These data suggest a relationship between daily doses of oral prescription medications and idiosyncratic DILI. More studies are needed to validate these observations and to explore their implications. (HEPATOLOGY 2008;47:2003-2009.)
Structural Alerts, Dose Size and Toxicity: Top 200 Most Prescribed Drugs in 2009

Top 20 most prescribed
• 10 (55%) contain structural alert(s)
• 5/10 (50%) are reactive metabolite positive and/or demonstrate covalent binding

Top 180 most prescribed
• 48 out of 93 drugs (51%) contain structural alert(s)
• 19/48 (39%) are reactive metabolite positive and/or demonstrate protein covalent binding

Structural alert type

Atorvastatin
RM +ve, covalent binding to HLM
Daily dose = 10 - 20 mg

Clopidogrel
RM +ve, covalent binding to HLM
RM is required for pharmacology
Daily dose = 75 mg (> 80% of CL is via hydrolysis)

Tadalafil
RM +ve, covalent binding to HLM
Daily dose = 5-20 mg

Paroxetine
RM +ve, covalent binding to HLM
Daily dose = 20-60 mg
Structural Alerts, Dose Size and Toxicity: Top 200 Most Sold Drugs in 2009

- 13/15 (86%) contain structural alert(s)
- 10/13 (77%) are reactive metabolite positive and/or demonstrate covalent binding

**Structural alert type**

- Injectible proteins
  - Pioglitazone: RM +ve, covalent binding to HLM, Daily dose = 15-45 mg
  - Duloxetine (Cymbalta): RM +ve (on napthyl ring), Daily dose = 60 mg

**Structural alert type**

- Pramipexole (Mirapex): Daily dose = 4.5 mg
- Erlotinib (Tarceva): RM +ve, Daily dose = 150 mg
Daily Dose Trends – Most Prescribed Drugs (2009)

- A key differentiator from the 68 toxic drugs is the daily dose
- 50% of the drugs are in the 10 – 50 mg dose range
- High dose drugs (> 100 mg) in the most prescribed list are HIV agents and antibiotics
A key differentiator from the 68 toxic drugs is the daily dose.

- ~ 50% of the drugs are in the 10 – 50 mg dose range.
- High dose drugs (> 100 mg) in the most prescribed list are HIV agents, antibiotics, and newer oncology drugs (kinase inhibitors).
- ~ 30% are biologics or topicals agents.
Influence of Daily Dose on Drug Toxicity

Amineptine
- RM +ve (aromatic epoxide)
- Inhibits fatty acid oxidation
- Hepatotoxic (withdrawn)
- Daily dose = 200 mg

Tianeptine
- RM +ve (aromatic epoxide)
- Inhibits fatty acid oxidation
- Non-hepatotoxic
- Daily dose = 37.5 mg

Troglitazone
- RM +ve (quinone-methide, thiazolidinedione ring scission)
- BSEP inhibitor (parent + sulfate metabolite)
- Mitochondrial toxin
- Hepatotoxic (withdrawn)
- Daily dose = 600 mg

Pioglitazone
- RM +ve (thiazolidinedione ring scission)
- BSEP inhibitor
- Non-hepatotoxic
- Daily dose = 15-45 mg
Concluding Remarks (Collation of Peer-Reviewed Publications)

- Origins of IADRs can be multifactorial
  - RM formation is one factor in the overall scheme of mechanism(s) leading to toxicity
    - Consider the other mechanism(s), which will be discussed in the course
    - Categorize IADR risks in a integrated fashion

- From a preclinical drug discovery perspective
  - Detection of RM (glutathione conjugates or protein covalent binding) is a hazard indicator and not necessarily a predictor of toxicity
    - But evidence for involvement of RM as a causative factor in toxicity is strong, particularly for drugs with a high daily dose (e.g., acetaminophen)
  - Recommended caution in the use of structural alerts in lead chemical matter, particularly if structural alert is susceptible to bioactivation
    - Reduce/eliminate RM liability in lead chemical matter, if pharmacologic SAR permits
      - Why take the chance, avoid unnecessary debate at development phase
    - Examine competing metabolism/detoxification pathways
    - Estimation of daily dose (based on projected human PK and efficacious circulating concentrations)
      - Particularly important when nominating a RM-positive compound as a drug candidate (See Pfizer Case Study – 11β-HSD1 Inhibitors in back-up material)
Back-up Material

- AZ ANALYSIS
- EXAMPLE OF STRUCTURE-METABOLISM-TOXICITY RELATIONSHIP
- PFIZER CASE STUDY
AZ pipeline analysis for attrition

Lessons learned from the fate of AstraZeneca’s drug pipeline: a five-dimensional framework

David Cook, Dearg Brown, Robert Alexander, Ruth March, Paul Morgan, Gemma Satterthwaite and Menelas N. Pangalos

Vol. 13 | June 2014 | 419
Structure-Metabolism-Toxicity Relationships

Circumstantial evidence that avoiding RM formation will eliminate toxicity

**Example:** *Imidazopyridine-based anxiolytic agents*

- **Alpidem**
  - Hepatotoxic (acute liver failure/fatalities)
  - Withdrawn within 1\(^{st}\) year of release into market

- **Zolpidem (Ambien\(^{®}\))**
  - Non-hepatotoxic
  - Top 200 list (most prescribed/most sold in 2009)
Differences in Metabolism of Alpidem and Zolpidem in Humans

- Metabolic route consistent with glutathione depletion in human hepatocytes
- Alpidem is a mitochondrial toxin

- No aryl ring epoxidation (no glutathione conjugates)
  - Pendant methyl groups on aryl rings are hydroxylated instead
  - Does not deplete GSH in human hepatocytes
- Zolpidem is not a mitochondrial toxin
- Zolpidem has a lower daily dose (5–10 mg) than alpidem (150 mg)

PF-0915275 was a lead candidate in the 11β-HSD1 inhibitor program

- Selective single digit nM inhibitor of 11β-HSD1
- Excellent predicted clinical pharmacokinetics
- PF-0915275 was devoid of structural alerts
  - But formed GSH adducts in HLM/NADPH/GSH

Proposed mechanism of bioactivation

- Compound intended to treat type 2 diabetes mellitus (non-life threatening indication with numerous existing therapeutic choices in the clinic)

Risk assessment was deemed necessary with additional SAR to eliminate RM liability
Risk Assessment with PF-0915275

• Significant reduction (almost to background levels) in covalent binding to liver S9 fraction upon inclusion of GSH and other phase 2 enzyme co-factors, implying detoxication of reactive metabolite precursor

• Upon oral administration to rats:
  • Very low covalent binding to rat liver protein (0.21 pmol/mg; < 0.05% of dose)
  • No GSH (or downstream mercapturic acid) conjugates detected in circulation, bile or urine

• Projected daily efficacious dose in humans
  • 0.6–3.0 mg based on animal pharmacology studies and projected human pharamacokinetics (CL, Vdss, oral F)

• Back-up compound devoid of bioactivation identified