

## Metabolites and Safety: Diligence on Safety – Expedience for Patients

New England Drug Metabolism Discussion Group  
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Cambridge, MA

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Slide 1 of 53

## Outline

1. History on MIST
2. A Conceptualization of Toxicity Mechanisms
3. Reactive Metabolites and Covalent Binding Assays
4. Challenges in Meeting the FDA Guidance  
MIST Conundrums
5. Conclusions

Slide 2 of 53

## First, Some Conclusions

*We are all after the same goal: New advances in pharmacotherapy that are safe and effective, provide acceptable benefit-to-risk ratios, and are brought to patients who need them with expediency and diligence.*

*We need to answer the right questions with the right experiments at the appropriate time.*

Slide 3 of 53

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Slide 4 of 53

## MIST History: How We in R&D Organizations Handled Metabolites in Drug Development

- During drug development two types of scenarios regarding metabolites frequently arose:
  - Scenario 1: Unexpected safety findings could be observed in the clinic
    - Triggered unwarranted speculation that there must be a human metabolite as the cause
    - This led to unproductive random searching for the “toxic metabolite”
  - Scenario 2: Metabolites were identified as part of the standard characterization of the properties of the drug
    - Triggered unwarranted notions that they could be toxic
    - This led to unneeded bioanalysis and testing

Slide 5 of 53

## PhRMA White Paper Was Developed

- Baillie, et al., Tox. Appl. Pharm. 2002
- Aimed to provide a framework for when to consider metabolites in safety
  - Would remove ambiguity and anxiety over metabolites
  - When to direct resource to the problem
- Scientifically driven
  - Metabolites as contributors to the whole (25% drug-related material)
  - Leveraged human radiolabel ADME study as the defining data
  - Considered chemical structure and our knowledge of importance of structure in toxicity
  - Only flaw – based the cutoffs on *relative* abundance not *absolute* abundance
    - ADME data historically reported in %
    - Metabolites of low dose drugs would receive extra unnecessary scrutiny
    - Metabolites of high dose drugs could evade necessary scrutiny
    - We know that high dose drugs tend to have more toxicity problems than low dose drugs

Slide 6 of 53

## FDA Counter-Opinion

- Initial draft guidance – dropped the cutoff to 10%
  - Based on previous guidances of this type
  - No consideration of structure
  - Confusing on % of dose, % of circulating material
- Final Guidance
  - Changed from % of total to % of parent (???)
  - Requires steady-state comparison across species

.....More on this later

Slide 7 of 53

## The Rationale for 10%

- Reflects consistency with other FDA and EPA regulatory guidances (U.S. Food and Drug Administration 2002; U.S. Environmental Protection Agency 1998) .
- Cases cited – ALL high dose **Reactive Metabolite** Examples
- **Halothane** (hepatotoxic) trifluoroacetylchloride intermediary reactive metabolite excreted as TFA, which represents less than 20 percent of the administered dose. **13000 umol**
- **Felbamate** (aplastic anemia and hepatotoxic) phenyl propenal intermediary reactive metabolite, excreted as mercapturic acid and alcohol (15% percent of felbamate excreted in urine) **600 umol**
- **Acetaminophen** (hepatotoxic) N-acetyl-p-benzoquinone imine (NAPQI), a reactive intermediate of acetaminophen, excreted in urine as thioether metabolites (at 9% of dose of acetaminophen). **12000 umol**
- **Cyclophosphamide** – intentionally toxic cancer chemotherapeutic

Slide 8 of 53

*Example 50 mg/day drug: 10% metabolite is 14 umol*

Before crafting a criteria for metabolites in safety, we need to ask:

Why this is important?  
What toxicity are we trying to understand?

Slide 9 of 53

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Slide 10 of 53

## Two Fundamental Types of Toxic Mechanisms

Stable Compound  
Reversible Interaction  
with Macromolecule

- Toxic molecule binds to a specific receptor or enzyme involved in a critical cellular or physiologic process
  - Exacerbation of target pharmacology
  - Off-target pharmacology

"Class A"

Park, Pirmohamed, and Kitteringham (1998) Chem. Res. Toxicol. 11: 969-988

*These are simple categorizations for which there are exceptions, but they help frame the thinking*

Reactive Compound  
Irreversible Interaction  
with Macromolecule

- Chemically reactive entity alters proteins, nucleic acids
  - General tissue effects (e.g. necrosis)
  - Immunoallergic
  - Mutation, Cancer

"Classes B-D"

Slide 11 of 53

## Two Fundamental Types of Toxic Mechanisms Possible Impact of Metabolites

Stable Compound  
Reversible Interaction  
with Macromolecule

- Chemically stable metabolites
- In circulation

Reactive Compound  
Irreversible Interaction  
with Macromolecule

- Chemically unstable metabolites
- Not in circulation
  - Downstream by-products in excreta

Slide 12 of 53

## Two Fundamental Types of Toxic Mechanisms Withdrawn or Black-Box Warning Drugs

Stable Compound  
Reversible Interaction  
with Macromolecule

Alosetron  
Cerivastatin  
Encainide  
Flosequinan  
Rofecoxib  
Astemizole  
Cisapride  
Dexfenfluramine  
Grepafloxacin  
Mibefradil  
Rapacuronium  
Terfenadine

Reactive Compound  
Irreversible Interaction  
with Macromolecule

Benoxaprofen  
Bromfenac  
Nefazodone  
Nomifensine  
Remoxipride  
Suprofen  
Terafloxacin  
Tolcapone  
Troglitazone  
Trovafoxacin  
Zomepirac

Slide 13 of 53

Blue: Stable Metabolite Involved Red: Reactive Metabolite Involved

## Two Fundamental Types of Toxic Mechanisms

Stable Compound  
Reversible Interaction  
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Reactive Compound  
Irreversible Interaction  
with Macromolecule

Acetaminophen  
Felbamate  
Halothane  
Cyclophosphamide

- These were the toxic drugs used in defining the 10% criteria
- Toxicity is not due to specific target and is due to **reactive metabolites**
- They do not have circulating toxic metabolites

Slide 14 of 53

## Two Fundamental Types of Toxic Mechanisms

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Reversible Interaction  
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Reactive Compound  
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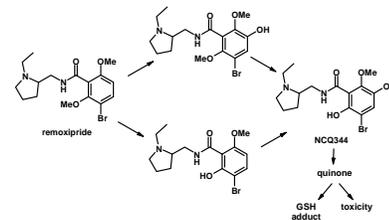
Among drugs, we could not find a single example of a case in which a circulating human-disproportionate metabolite was the cause of a toxicity derived from a specific interaction that was not already an activity possessed by the parent drug

Acetaminophen  
Felbamate  
Halothane  
Cyclophosphamide

- But the present guidance is focused on circulating metabolites that are 10% of parent
- Due to its focus on circulating metabolites, the guidance over-emphasizes those that possibly could cause toxicity by a interaction with a **specific target**

Slide 15 of 53

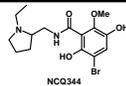
## Remoxipride Example



- Antipsychotic agent used in Europe in 1990s
- Aplastic anemia at 1 in 50000; withdrawn
- In vitro studies showed generation of hydroquinone and GSH adduct

Slide 16 of 53

## Remoxipride



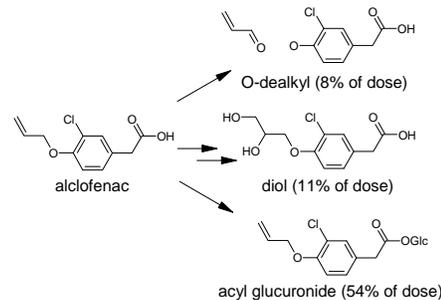
- Remoxipride is a rare example of analysis of plasma samples for a metabolite involved in reactive metabolite generation
- Human plasma assay for NCQ344
  - Detected 0.1 to 1.0 nM in patient samples
  - Compares to remoxipride at 2500-20000 nM
- Precursor phenol metabolite and its conjugates present in human excreta; comprised 0.6% (0.45 mg)
- In animals, phenol+conjugates was much greater than in humans
  - Shows that even when metabolite(s) is "covered" in animals human safety problems can still occur

Slide 17 of 53

Ref. Widman, et al., Arz Forsch 43, 287, 1993; Nilsson, Biomed Chrom. 12, 65, 1998; Erve, et al., Chem Res Tox 17, 564, 2004

## Excretory Metabolites and Drug Withdrawals: Alclofenac

- Alclofenac: NSAID drug for rheumatoid disease



Slide 18 of 53

### Excretory Metabolites and Drug Withdrawals: Alclofenac

- Dosed at 1 gm/day
- Diol was 11% of excreted dose (~110 mg)
  - But was >50% of dose in rodents: covered (?)
- Diol would have obligatory epoxide intermediate
  - Epoxide actually measured in human urine
  - 172 ug (after 3 gm dose) was measured (0.0057%)
  - No report of a GSH adduct or mercapturic acid
  - Unusual example: reactive intermediates not typically measurable
- O-dealkylation yields intermediary acrylaldehyde
  - 8% in human; 30% in rodents
- Acyl Glucuronide
  - major in human and monkey (>60%); minor/trace in rodents
- Withdrawn for skin rash (30%)
  - What metabolite monitoring would have yielded an understanding of this?

Slide 19 of 53

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Slide 20 of 53

### Assessing Safety of Metabolites: Reactive Metabolites

- An analysis of 24 withdrawn drugs showed that animals had body burdens for reactive metabolites (or systemic exposures for stable metabolites) in excess of that in humans
  - Therefore, no further testing of metabolites in animals would be expected to yield insight into human toxicity
- Maybe this is a function of conventional animal toxicology studies having limitations for predicting human effects, and not because of differences in metabolism

Smith and Obach, Chem Res Tox 19, 1570, 2006

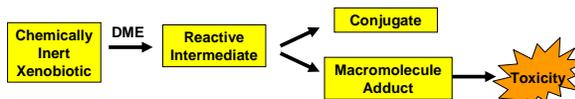
Slide 21 of 53

### Assessing Safety of Metabolites: Reactive Metabolites

- This is a difficult problem
- Measurement of metabolites that are 10% of parent in *human circulation*, as described in the FDA Guidance, will do nothing to address the issue of reactive metabolites
- Maybe there is a *body-burden* threshold for reactive metabolites?
- Is there anything we can do with human-derived in vitro systems?

Slide 22 of 53

### What About In Vitro Approaches to Reactive Metabolites?



- This concept has been established for over 50 years
  - Carcinogenesis and Mutagenesis
  - Protein adducts and toxicity
- Retrospective testing of in vitro covalent binding to explain toxicity has been done for many compounds
- Some have advocated using:
  - Nucleophile adduct assays
  - Covalent binding assays to make toxicity predictions

Slide 23 of 53

### In Vitro Covalent Binding - Approach

- Experiment:
  - Microsomes + radiolabelled drug +/- NADPH
  - Precipitate the protein, wash, solubilize and count the pellet
- This has been done for many known hepatotoxins (e.g. APAP, diclofenac, etc) but not for non-toxins
- High profile publications touting these experiments as part of a drug selection process
  - "50 pmol/mg protein" (Evans, et al., Chem Res Tox, 2004)
- Potential flaws in this approach
  - No consideration of dose
  - No consideration of rate of metabolism
  - No consideration of detoxication mechanisms
  - No consideration of relative importance of bioactivation pathway to total metabolism
  - Confuses rate and extent

Slide 24 of 53

## What About In Vitro Approaches to Reactive Metabolites?

- Covalent binding assays have not been rigorously tested across toxic vs non-toxic compounds
- Objectives:
  - Test human microsomal covalent binding assay across 18 hepatotoxins and non-hepatotoxins
  - Obtained 18 radiolabelled drugs
  - Utilize an intrinsic clearance approach ( $CL_{int}$ )
  - Does covalent binding  $CL_{int}$  separate toxins from non-toxins?
  - How does the % of total  $CL_{int}$  comprised by covalent binding  $CL_{int}$  influence this?
  - Is there an impact of total daily dose?

Chem Res Toxicol, ASAP article

Slide 25 of 53

## Test of Human Liver Microsomal Covalent Binding

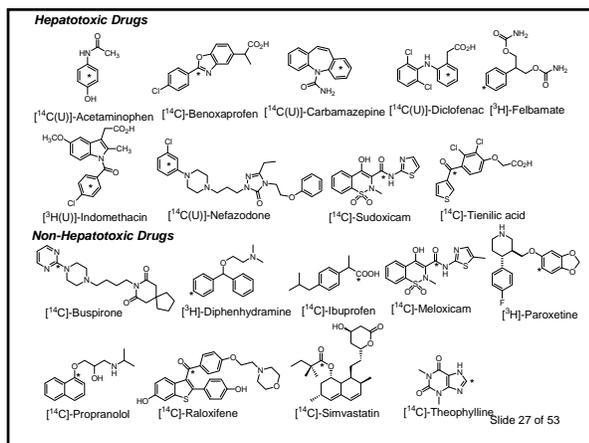
### The Hepatotoxins:

Acetaminophen  
Benoxaprofen  
Carbamazepine  
Diclofenac  
Felbamate  
Indomethacin  
Nefazodone  
Sudoxicam  
Tienilic Acid

### The Non-Hepatotoxins:

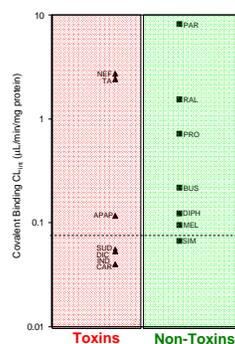
Buspirone  
Diphenhydramine  
Ibuprofen  
Meloxicam  
Paroxetine  
Propranolol  
Raloxifene  
Simvastatin  
Theophylline

Slide 26 of 53



Slide 27 of 53

## Test of Human Liver Microsomal Covalent Binding



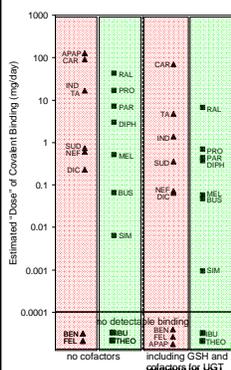
Seven of nine in each category showed covalent binding

No binding for theophylline, ibuprofen, felbamate, benoxaprofen

Non-Toxins showed equal or greater covalent binding activity than toxins

Slide 28 of 53

## Test of Human Liver Microsomal Covalent Binding



$$\text{Dose of Covalent Binding} = \text{Daily Dose} \cdot \frac{CL_{int,cb}}{CL_{int,cb} + CL_{int}}$$

Include dose and fraction of in vitro  $CL_{int}$  comprised by covalent binding

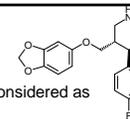
Uncorrected and corrected for impact of GSH and glucuronidation

In vitro human liver microsomal covalent binding still does not distinguish hepatotoxins from non-toxins

Covalent binding is probably best used to explore mechanisms, but not to predict or risk-assess toxicity

Slide 29 of 53

## Some Interesting Cases: Paroxetine



- Paroxetine is a very widely used antidepressant considered as non-hepatotoxic
- Structure has a methylenedioxy, a known structural alert
  - Bioactivation to carbene which can covalently bind
  - Metabolism to catechol, which can be oxidized to o-quinone and bind
  - 14-19% of total metabolism is covalent binding
- Covalent binding is high; 14-19% of total metabolism
- GSH and active methyltransferase reduce binding by over 5X
- Typical dose is 20 mg/day; high dose is 50 mg/day
- Conclusions:
  - Exemplifies low dose compound – can tolerate higher in vitro covalent binding
  - Detoxication pathways are important to include
  - Simple in vitro covalent binding approach would have led to discarding paroxetine
  - Additional efforts have led to the characterization of paroxetine GSH adducts (Kalgutkar, et al; recently published in CRT)

Slide 30 of 53

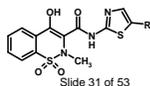
### Some Interesting Cases: Sudoxicam vs Meloxicam

- Sudoxicam: withdrawn from development in the 1970s due to severe hepatotoxicity
- Meloxicam: successful NSAID; not considered hepatotoxic
- Both possess 2-aminothiazole, a structure alert; meloxicam has additional 5-methyl

Covalent binding CL <sub>int</sub> (uL/min/mg)	sudoxicam	meloxicam
No GSH	0.056	0.095
With GSH	0.026	0.012
% of total consumption	1.5	3.4
Total Daily Dose (mg)	50	15
Covalent Binding "Dose" (No GSH)	0.75	0.51
Covalent Binding "Dose" (+GSH)	0.34	0.066

#### Factors(?)

- Lower dose of meloxicam
- Greater impact of GSH on meloxicam; observation of unique GSH reduced metabolite for meloxicam but not sudoxicam



Obach et al. 2008, in press

### Assessing Safety of Metabolites: Reactive Metabolites

- So, what can we do????
  - Avoid structure alerts whenever possible and as early as possible
  - Identify proclivity for reactive metabolites early in drug discovery, when medicinal chemists still have a chance to re-design lead molecules
    - "Yes/No" answer will have to suffice
    - Maybe quantitative comparisons within a close-in series can be done?
  - Keep the dose low
    - Keeps body burden of foreign matter to a minimum
    - Seek/design compounds of high potency and low unbound clearance

Slide 32 of 53

### Assessing Safety of Metabolites: Reactive Metabolites

- And what is not worth it?
  - Quantitative comparisons of in vitro covalent binding
  - Assuring animal "coverage" for reactive metabolites
    - Cannot study enough animals to model human toxicities caused by reactive metabolites
    - We don't know why

Slide 33 of 53

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Slide 34 of 53

### Now, Back to Circulating Metabolites and Guidance

- Positive Aspects FDA MIST Guidance
  - Reflects FDA *current thinking* on a topic and viewed as *recommendations*
  - Industry currently operates in good alignment with most recommendations in guidance
  - For serious or life-threatening diseases or unmet medical need, the number and type of nonclinical studies for metabolites can be modified.
  - Metabolite exposure needs to exceed humans in only one animal test species.
  - With a few exceptions, phase II conjugation reactions generally eliminate the need for further evaluation of conjugate

Slide 35 of 53

### Circulating Metabolites and Guidance

- Changes in FDA Guidance
  - Metabolites that are 10% of circulating parent drug need to be studied further
    - From a toxicological standpoint this would only make sense if the safety concern for metabolites were the same as parent
    - Increases metabolites of concern from a median of one per drug to three per drug
    - Does not address reactive metabolites, which were the initially cited examples
  - Requires assessment of steady-state exposure comparison between humans and toxicology species
    - Cannot use single dose radiolabel ADME data to make the comparison
    - Requires development of assays for anything in human that is >10% of parent in the radiolabel study
      - Maybe there are ways to get the information without formally developing an assay for everything?

Slide 36 of 53

## MIST Conundrums

- What do we do if our parent compound is very potent?
  - Metabolites of “concern” could be present at very low concentrations and not cause toxicity
- What do we do when our parent compound is highly metabolized and represents a negligible percentage of total drug-related material in circulation?
  - How many metabolites do we need to qualify? Is there a limit?
- How do we make these assessments at steady-state when we cannot be doing steady-state radiolabel studies?
  - Conventional assays developed for multiple metabolites per drug?

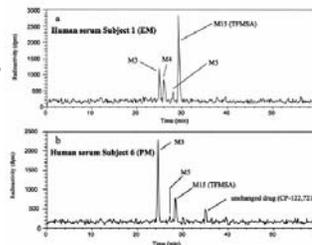
*The burden around MIST has shifted from toxicology groups to the groups that identify metabolites and develop, validate, and conduct bioanalytical methods for quantitation of metabolites in biological samples (DMPK groups).*

Slide 37 of 53

## Extensive Parent Metabolism Conundrum:

Metabolite/Parent AUC = “Infinity Percent”

- When human plasma radiochromatograms reveal extensive parent metabolism:
  - A M/P denominator of zero puts all metabolites at “infinity percent”
  - Many metabolites to synthesize and develop assays for
  - Odds of a disproportionate metabolite higher



Karnel, et al., DMD, 2007

Slide 38 of 53

TABLE 2

Relative percent distribution of radioactive metabolites in pooled plasma, urine, and fecal samples after p.o. administration of [<sup>14</sup>C]dasatinib to humans

Metabolite ID	% Distribution					
	Plasma (2 h)		Urine (0-168 h)		Feces (0-168 h)	
	% Radioactivity	% Dose	% Radioactivity	% Dose	% Radioactivity	% Dose
M3a,b <sup>d</sup>	3.3	6.8	0.2	N.D.	N.D.	
M4	MS	1.3	0.05	3.1	2.6	
M5	4.5	39.8	1.4	N.D.	N.D.	
M6	3.6	1.3	0.05	10.4	8.9	
M7	3.3	2.1	0.08	N.D.	N.D.	
M8a	3.4	5.5	0.2	N.D.	N.D.	
M8b, M23a,b <sup>d,e</sup>	1.4	N.D.	N.D.	N.D.	N.D.	
M9	N.D.	N.D.	N.D.	1.8	1.5	
M20	12.5	4.1	0.2	36.6	31.2	
M21	9.5	7.8	0.3	N.D.	N.D.	
M23a,b	N.D.	N.D.	N.D.	14.7	12.5	
M24	3.1	6.0	0.2	4.7	4.0	
M30	6.9	N.D.	N.D.	N.D.	N.D.	
M31	3.6	N.D.	N.D.	N.D.	N.D.	
M34	1.1	2.3	0.08	N.D.	N.D.	
M35a	3.6	4.2	0.2	N.D.	N.D.	
M36	N.D.	4.4	0.2	N.D.	N.D.	
M37a,b <sup>d</sup>	4.1	2.5	0.1	N.D.	N.D.	
Dasatinib	89.4	3.6	0.1	22.4	19.1	
Total		91.7	3.4	93.7	79.8	

Drug Metab. Dispos. 2008

Slide 39 of 53

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M8a	3.4	5.5	0.2	N.D.	N.D.	
M8b, M23a,b <sup>d,e</sup>	1.4	N.D.	N.D.	N.D.	N.D.	
M9	N.D.	N.D.	N.D.	1.8	1.5	
M20	N.D.	4.1	0.2	36.6	31.2	
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M23a,b	N.D.	N.D.	N.D.	14.7	12.5	
M24	3.1	6.0	0.2	4.7	4.0	
M30	6.9	N.D.	N.D.	N.D.	N.D.	
M31	3.6	N.D.	N.D.	N.D.	N.D.	
M34	1.1	2.3	0.08	N.D.	N.D.	
M35a	3.6	4.2	0.2	N.D.	N.D.	
M36	N.D.	4.4	0.2	N.D.	N.D.	
M37a,b <sup>d</sup>	4.1	2.5	0.1	N.D.	N.D.	
Dasatinib	89.4	3.6	0.1	22.4	19.1	
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Drug Metab. Dispos. 2008

Slide 40 of 53

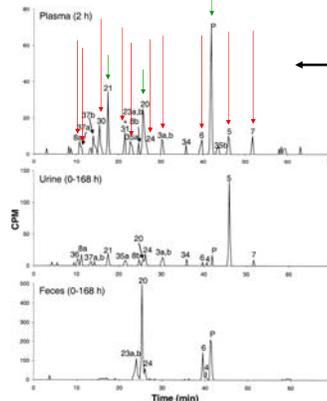
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M20	N.D.	4.1	0.2	36.6	31.2	
M21	N.D.	7.8	0.3	N.D.	N.D.	
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M30	6.9	N.D.	N.D.	N.D.	N.D.	
M31	3.6	N.D.	N.D.	N.D.	N.D.	
M34	1.1	2.3	0.08	N.D.	N.D.	
M35a	3.6	4.2	0.2	N.D.	N.D.	
M36	N.D.	4.4	0.2	N.D.	N.D.	
M37a,b <sup>d</sup>	4.1	2.5	0.1	N.D.	N.D.	
Dasatinib	89.4	3.6	0.1	22.4	19.1	
Total		91.7	3.4	93.7	79.8	

Compare to: in vitro potency = 0.4-5 nM; in vivo potency = 64 nM  
Clin. Canc. Res. (2006) and J. Med. Chem. (2004)

Slide 41 of 53



This is the radiometric HPLC data from which these percentages arise..... VERY LOW AMOUNTS

Drug Metab Dispos. (2008)

Slide 42 of 53

## Potent Drug Conundrum

- Low parent concentration or potent molecules
  - Pushes limits of all but accelerator mass spectrometry technology for plasma profiles
    - Miniscule metabolite mass-based risk but maximal cost, technical complexity and workload
    - Is HPLC-AMS the new standard for potent molecules? (And is it ready?)
- How to manage the worst case?
  - When potent parent is also extensively metabolized

Slide 43 of 53

## Steady-State Conundrum

- Human plasma  $^{14}\text{C}$  metabolite quantitation has many analytical caveats
  - Still the best way to observe exposure to "all" human metabolites
- Human  $^{14}\text{C}$  trials are **not** done at  $^{14}\text{C}_{\text{SS}}$ 
  - $^{14}\text{C}$  pulses at cold drug steady state creates variable specific activity across metabolites and parent – not appropriate
- Is a labor intensive new paradigm necessary to define clinical and preclinical steady state exposures?
  - Synthesize and qualify single dose human plasma  $^{14}\text{C}$  metabolites >10% parent AUC
    - Validate assay in all species
    - Apply assays human trial at steady state and in GLP multiple dose toxicology studies
    - Construct exposure multiples
    - Conduct safety investigations on all disproportionate metabolites
- Significant workload & cost required for a diligence exercise to rule out disproportionate metabolites definitively in early development
  - Are modelling approaches to metabolite/parent SS adequate?
  - AUCs requisite should be scrapped unless single dose metabolite half life is multiples of parent half life and dosing interval

Slide 44 of 53

## Are There Reasonable Solutions?

- Can we assure that human metabolites were present in excess in toxicology animals without synthesizing and developing GLP assays for multiple metabolites?

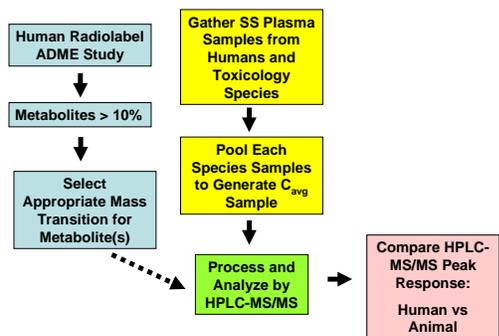
Slide 45 of 53

## One Idea

- Cross-species comparison of metabolite peak intensities
  - From radiolabel human ADME study, know which metabolites are present at >10% of parent
    - Know HPLC characteristics and MS fragmentation pattern
  - Gather steady-state human and toxicology species plasma samples
  - Make "AUC pools" (a la Hamilton, et al, Clin Pharmacol Ther, 29, 408, 1981)
  - Side-by-side work-up of animal and human samples
  - Inject on HPLC-MS, monitor MRM ion current channels for metabolites of interest
  - Compare peak areas; if peaks in animals are greater than in humans, then metabolite is covered
- There are other ideas

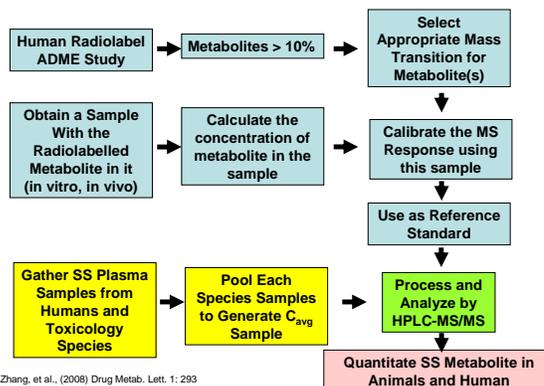
Slide 46 of 53

### Comparing Metabolites Across Species – Alternate Approaches 1. Steady-State MS Peak Area Comparison



Slide 47 of 53

### Comparing Metabolites Across Species – Alternate Approaches 2. Radiolabelled Calibrant



Zhang, et al., (2008) Drug Metab. Lett. 1: 293

## Outline

1. History on MIST
2. A Conceptualization of Toxicity Mechanisms
3. Reactive Metabolites and Covalent Binding Assays
4. Challenges in Meeting the FDA Guidance  
MIST Conundrums
5. **Conclusions**

Slide 49 of 53

## Conclusions

- Metabolites can cause safety concerns by
  - Specific interactions with specific targets (on- or off-)
  - Non-Specific reactions with essential biological macromolecules
  - However, animals will have exposures to human metabolites so failures of drugs due to human toxicity of metabolites may be due to shortcomings of animal models, not failure to adequately test metabolites in safety studies
- In vitro covalent binding studies do not predict toxicity
  - Only useful as a mechanistic tool for compounds already shown to be toxic
  - Further research is needed (e.g. specific protein targets?)

Slide 50 of 53

## Conclusions

- As written, FDA MIST guidance suffers from some shortcomings and may have created a need for extensive efforts that will not improve human safety of drugs.
  - MIST conundrums arise; difficult to satisfy for little or no return
  - Need to develop approaches that will add confidence regarding metabolite exposures without slowing the development of new therapies
  - The focus only on circulating metabolites may lead to overlooking reactive metabolites that can frequently contribute to toxicity

Slide 51 of 53

## Conclusions

*We are all after the same goal: New advances in pharmacotherapy that are safe and effective, provide acceptable benefit-to-risk ratios, and are brought to patients who need them with expediency and diligence.*

*We need to answer the right questions with the right experiments at the appropriate time.*

Slide 52 of 53

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Slide 53 of 53