DILIsym®

DILIsym Training Session 10:

Reactive Metabolites in DILIsym®

January 7, 2016

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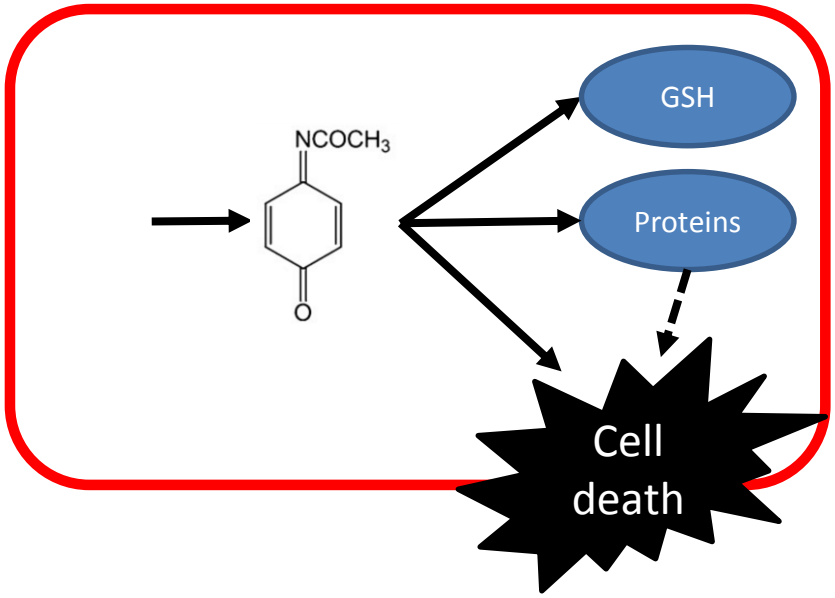
Please note: this presentation, including questions from the audience, is being recorded

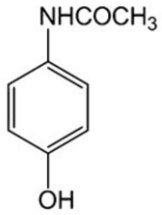
Reactive Metabolites in DILIsym®
Review Session Agenda

- Reactive Metabolite Generation in DILIsym®
- Fate of Reactive Metabolites in DILIsym®
- Reactive Metabolite Examples

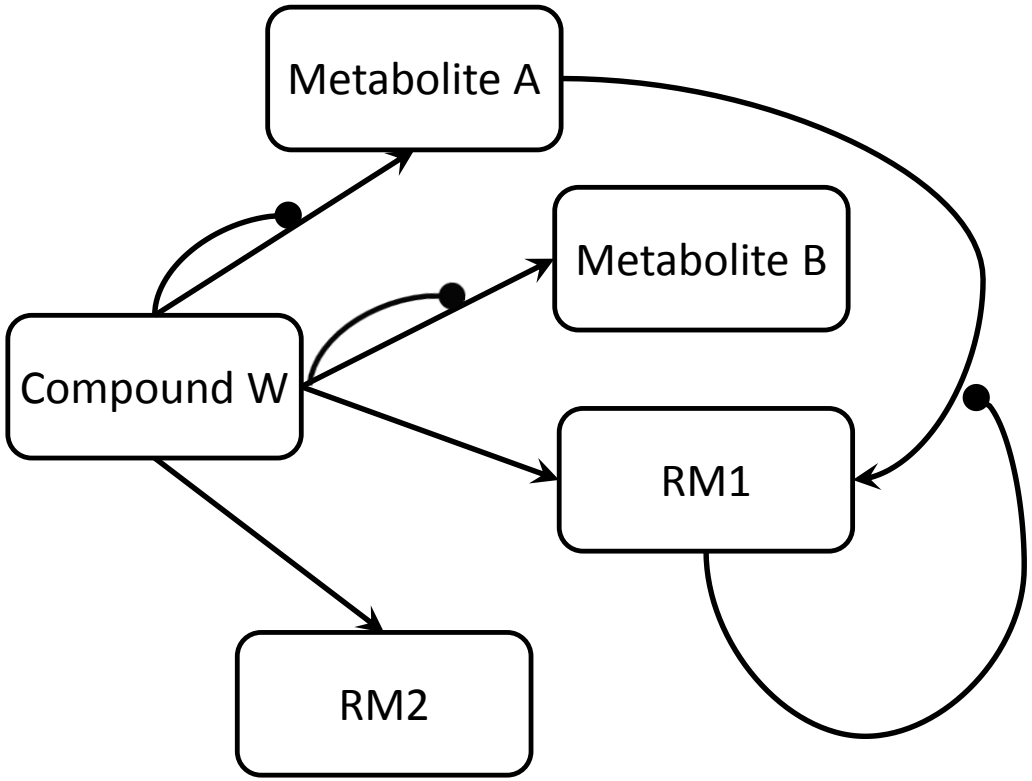
Reactive Metabolite-Mediated DILI

- Reactive metabolites (RM) are extremely labile metabolites generated in the liver that can covalently bind to cellular proteins or cellular antioxidants
- Ability to cause toxicity determined by amount of reactive metabolite generated and ability of the reactive metabolite to cause cellular stress
  - Reactive metabolites often, but not always, cause oxidative stress
  - Reactive metabolites themselves or adducts to cellular proteins can cause toxicity
- Examples of drugs that have reactive metabolites
  - Acetaminophen
  - Furosemide
  - Cocaine
  - Methapyrilene



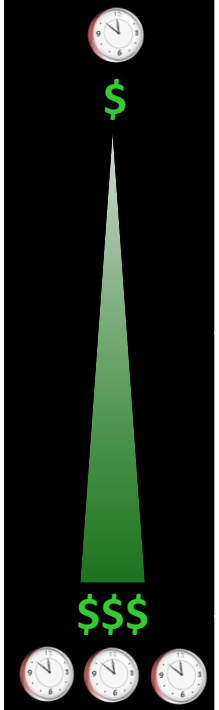


Compound Metabolism Representation in DILIsym® Includes Two Reactive Metabolites



- DILIsym® metabolic scaffold involves four potential metabolic pathways
  - Two stable metabolites and two reactive metabolites
- Reactive metabolites can be generated from parent compound or from Metabolite A
- Metabolism to RM values are difficult to calculate directly
  - Usually need information on RM fate (GSH depletion, protein adducts) from microsomal data in order to properly determine RM metabolism

Input Panel Document Provides Insight into Data Needed for RM Determination



Inputs or Data Needed	Commonly Used Systems/Assays	DILIsym® Preferred Systems
Concurrent GSH depletion, parent compound disappearance (and metabolite appearance if possible), protein adduct formation,	Primary hepatocytes; immortalized cell lines transfected with cytochrome P450s or other metabolising enzymes; ^{14}C or ^{3}H label for protein adducts	Primary hepatocytes; parent compound should be measured in the cells if possible, but can be estimated if not; comparisons to immortalized cells lines (HepG2) are useful from a metabolite perspective; data from cell lines transfected with drug metabolising enzymes are useful when available
ATP depletion	Primary hepatocytes; immortalized cell lines transfected with cytochrome P450s or other metabolising enzymes; whole-cell ATP	Primary hepatocytes; comparisons to immortalized cells lines (HepG2) are useful from a metabolite perspective; data from cell lines transfected with drug metabolising enzymes are useful when available
ROS/RNS indicators	Primary hepatocytes; immortalized cell lines transfected with cytochrome P450s or other metabolising enzymes; TBARS; peroxynitrite; lipid hydroperoxide, fluorescent probes (DCFDA, DHR123);	Primary hepatocytes; comparisons to immortalized cells lines (HepG2) are useful from a metabolite perspective; data from cell lines transfected with drug metabolising enzymes are useful when available

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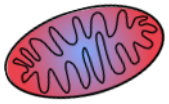
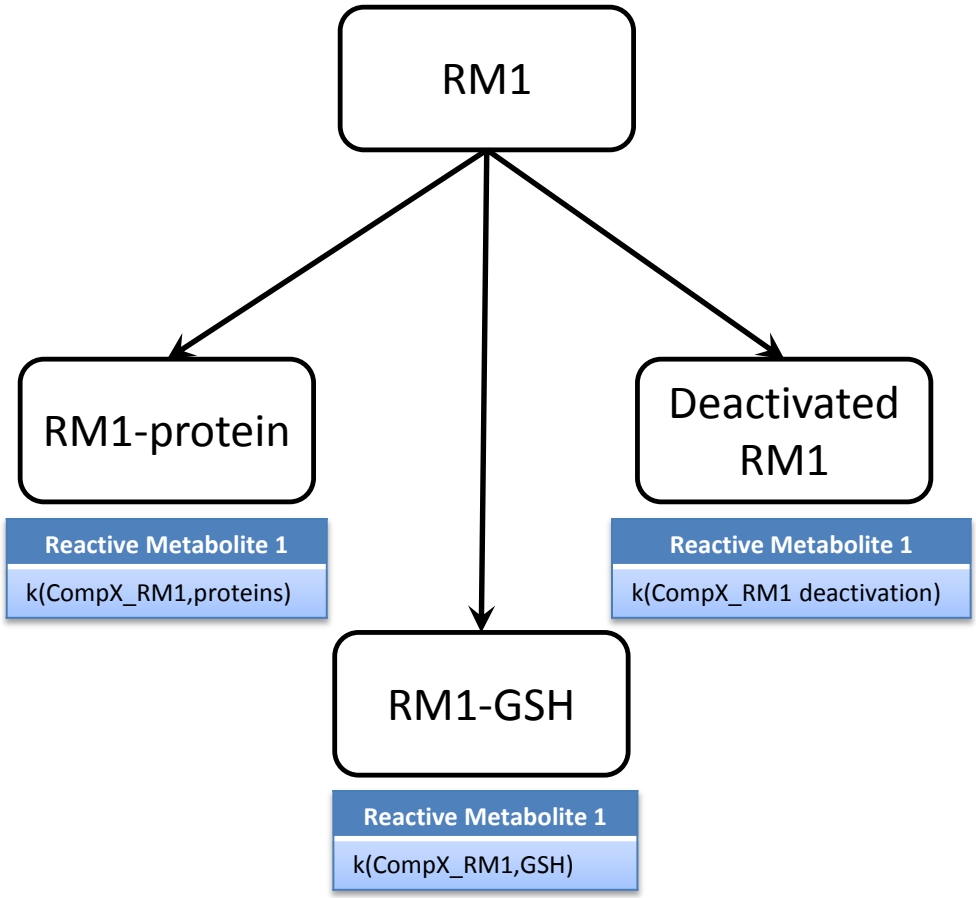


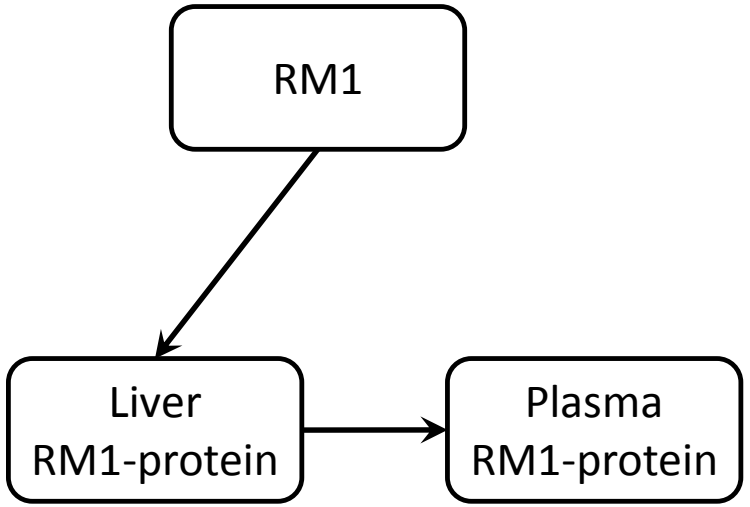
Reactive Metabolites Can Be Cleared in Several Ways

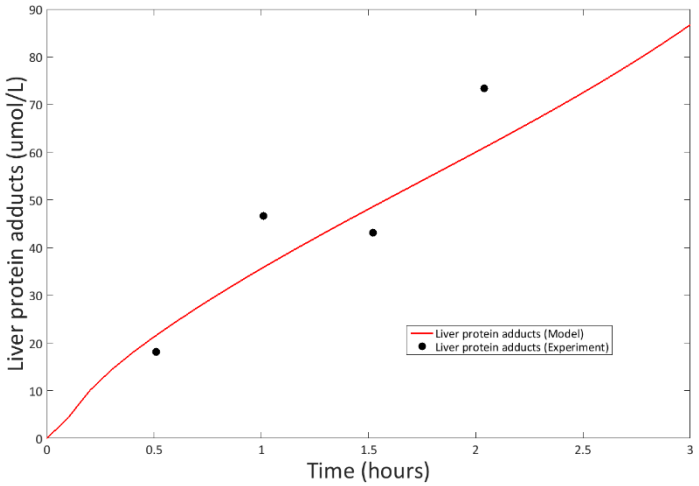


- Reactive metabolites have three potential modes of clearance
  - Adduction to cellular proteins
  - Conjugation with glutathione
  - Deactivation
  - Each is represented by a first-order process relative to the RM
- *In vitro* data determine which method(s) of clearance to model
  - GSH trapping assay can determine whether GSH conjugation is occurring
  - Protein binding is generally present with reactive metabolites
  - Deactivation occurs when the reactive metabolite is an intermediate; downstream metabolites will appear in a microsomal study

Protein Adducts in DILIsym®

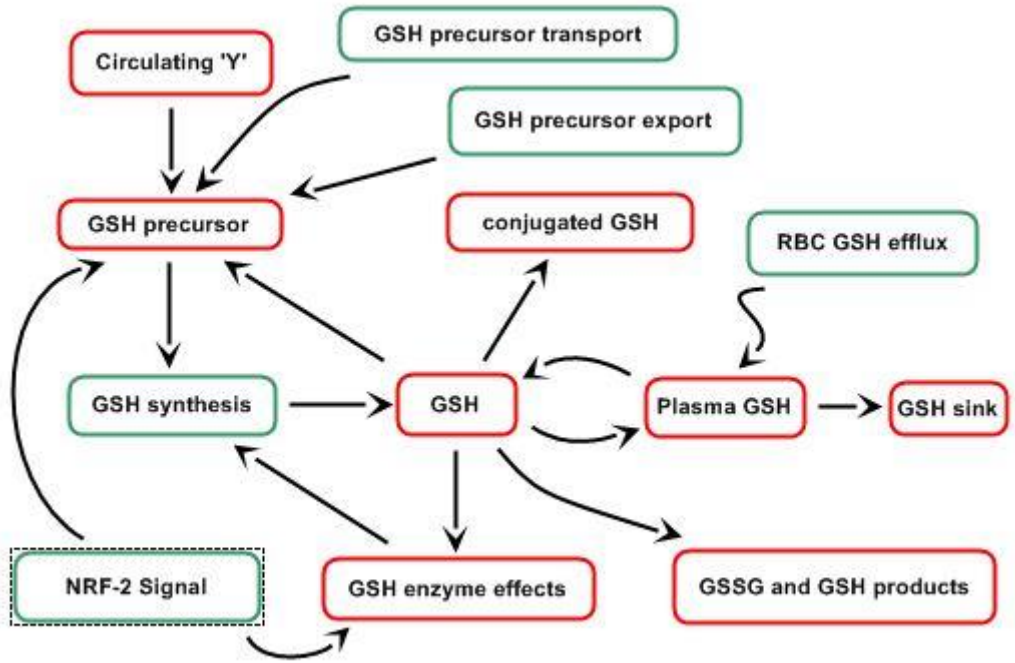
- Protein binding is a first-order process
- Protein adducts can cause toxicity
  - All DILIsym® toxicity mechanisms available to protein adducts
  - Adducts do not cause ROS in current APAP model
- Protein adducts are actively transported out of the hepatocyte
  - Adducts cleared from plasma by half-life
- Adducts can serve as a biomarker for validation
  - Example: APAP adducts in rat liver validated with data from Speeg 1985





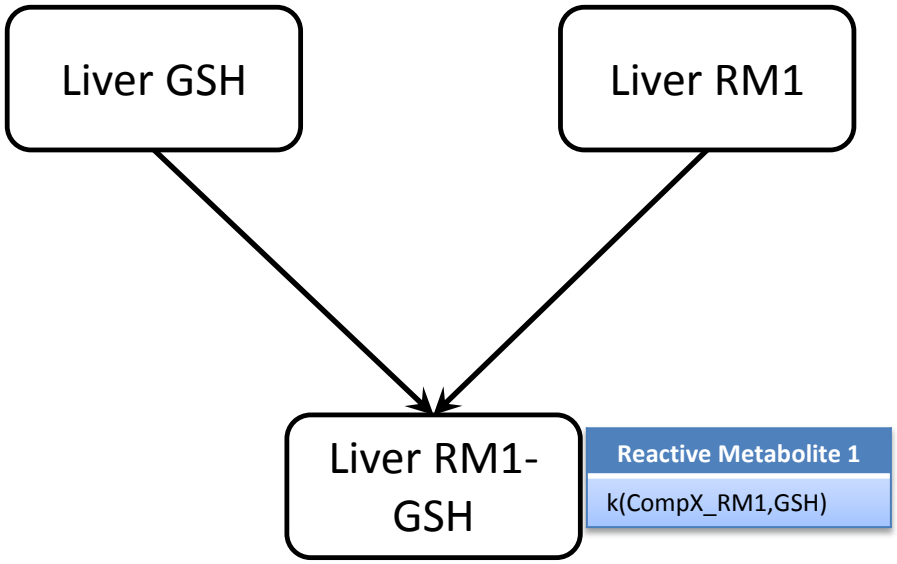
Glutathione (GSH) Model in DILIsym®

- DILIsym contains a model of GSH homeostasis and conjugation to reactive metabolites
  - Will be explored more in depth in future review sessions
- In DILIsym®, GSH exists only for conjugation by reactive metabolites
  - GSH is not affected by cellular oxidative stress from other sources
- Baseline GSH can vary across a population
  - Important for susceptibility to RM-mediated toxicity
- N-acetylcysteine can serve as a GSH precursor, boosting GSH levels
  - Uses existing Compound Y scaffold



GSH Binds and Neutralizes Reactive Metabolites

- RM-GSH reaction is second-order (first order with respect to both molecules)
  - Allows GSH depletion to occur
- RM-GSH is **not** available to cause toxicity
- Rate constant is extremely high in APAP case in order to reflect immediate reaction
  - Other RMs might not conjugate with GSH as readily
  - Rate constant can be determined by using *in vitro* GSH binding assays if expected to be in competition with other RM reactions *in vivo*



Reactive Metabolite Clearance Assumptions

- Reactive metabolites exist in the liver only
  - RM scaffold cannot be used to represent stable metabolites that require plasma tracking
  - Upon cell death, intracellular RM turns into plasma RM protein adducts
- Deactivated RM and RM-GSH are not cleared from the liver
  - Cumulative amount of each produced on a per-cell basis is calculated over the course of the simulation
  - Not released into the blood stream after cell death

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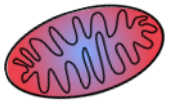
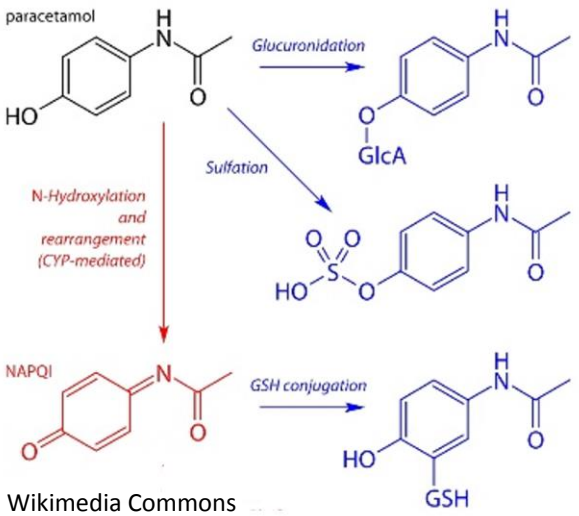


Acetaminophen Toxicity is Represented Extensively in DILIsym®

- Acetaminophen (APAP) is the drug most commonly implemented in DILI in the US and Europe
- APAP is metabolized into three metabolites, including NAPQI, which is reactive
  - Significant species differences in metabolism
- GSH quenches NAPQI easily; toxicity occurs when GSH is depleted and NAPQI can accumulate, causing oxidative stress
- Oxidative stress model in DILIsym® was calibrated using APAP data in rats, mice, and humans
  - Immune response model also frequently leverages data from the APAP literature



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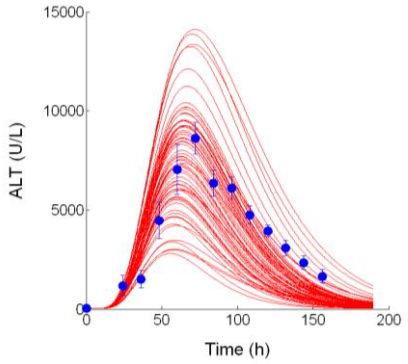
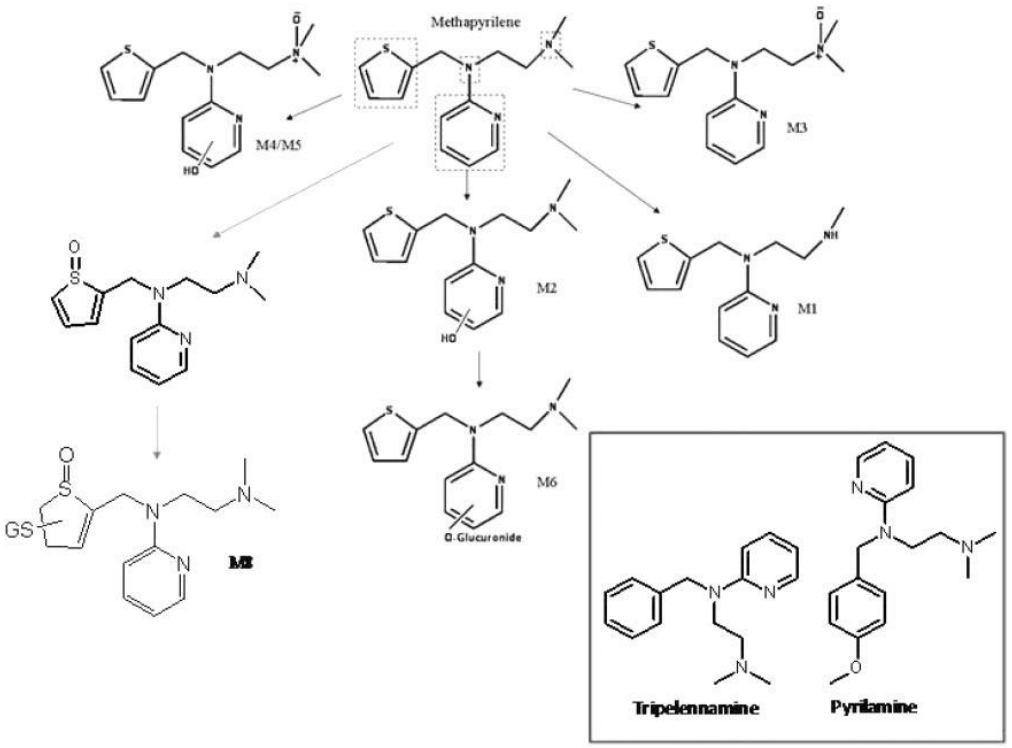


Representing Methapyrilene Requires Combining Several Metabolites from Microsome Studies

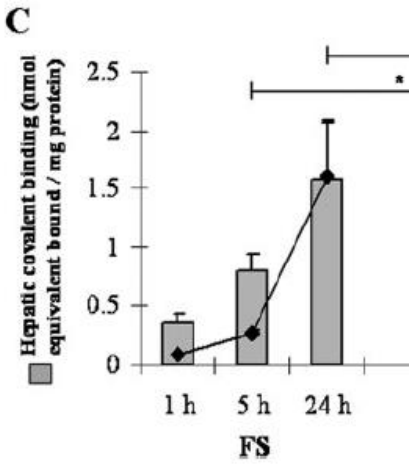
- Graham 2008 contains microsome metabolism data used to parameterize methapyrilene metabolism parameters
  - Percentage of initial incubation of MP transformed into various metabolites was reported
- V_{max} values are determined by calculating the values of V_{max} that produce the appropriate percentages after the correct amount of time
  - K_m values are assumed from reported values for similar metabolism pathways
  - K_m could be calculated using microsome incubations at different concentrations; in practice, these data rarely exist
- GSH depletion dynamics similar to acetaminophen
  - Can assume same GSH binding rate constant



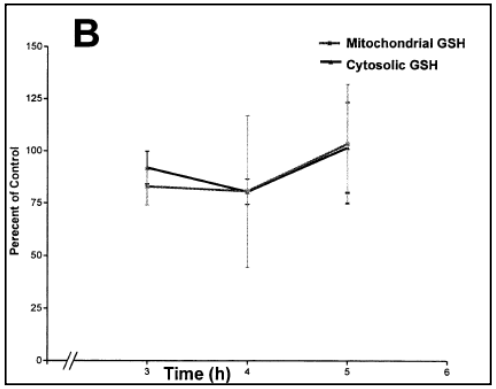
Graham JPET 2008

Furosemide Has a Reactive Metabolite But Does Not Deplete Glutathione

- Furosemide causes hepatotoxicity in mice at high doses
- Covalent binding in mouse studies implicates a reactive metabolite
  - Microsomal metabolism data backs this up
- GSH is not depleted in furosemide-treated mice
  - Reactive metabolite binds to proteins but not GSH
  - Also suggestive of a toxicity mechanism other than oxidative stress (in this case, increased ATP utilization)



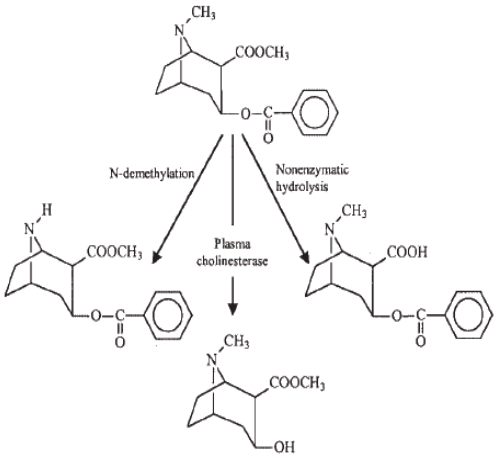
Williams, JPET 2007; CD-1 mice, 400 mg/kg

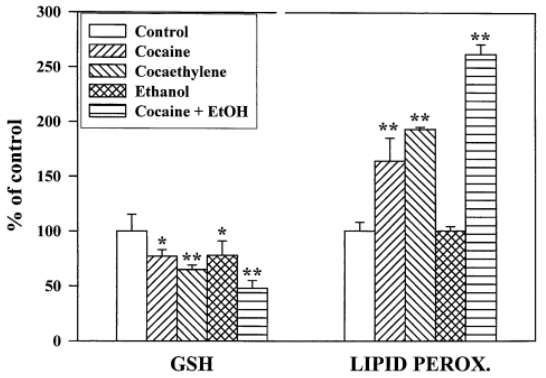


Wong Tox Letters 2000; Swiss CD-1 mice, 400 mg/kg

IVIVE with Cocaine Requires Use of *In Vitro* Lipid Peroxidation and GSH Data

- Cocaine is metabolized to three main metabolites, one of which is reactive
  - Increase in lipid peroxidation suggests N-demethylated metabolite causes oxidative stress
- GSH assay shows some mild depletion *in vitro*, suggesting weaker GSH binding than APAP
  - Microsomal data is less abundant, so a lower GSH binding constant than the one used for APAP/MP can be assumed

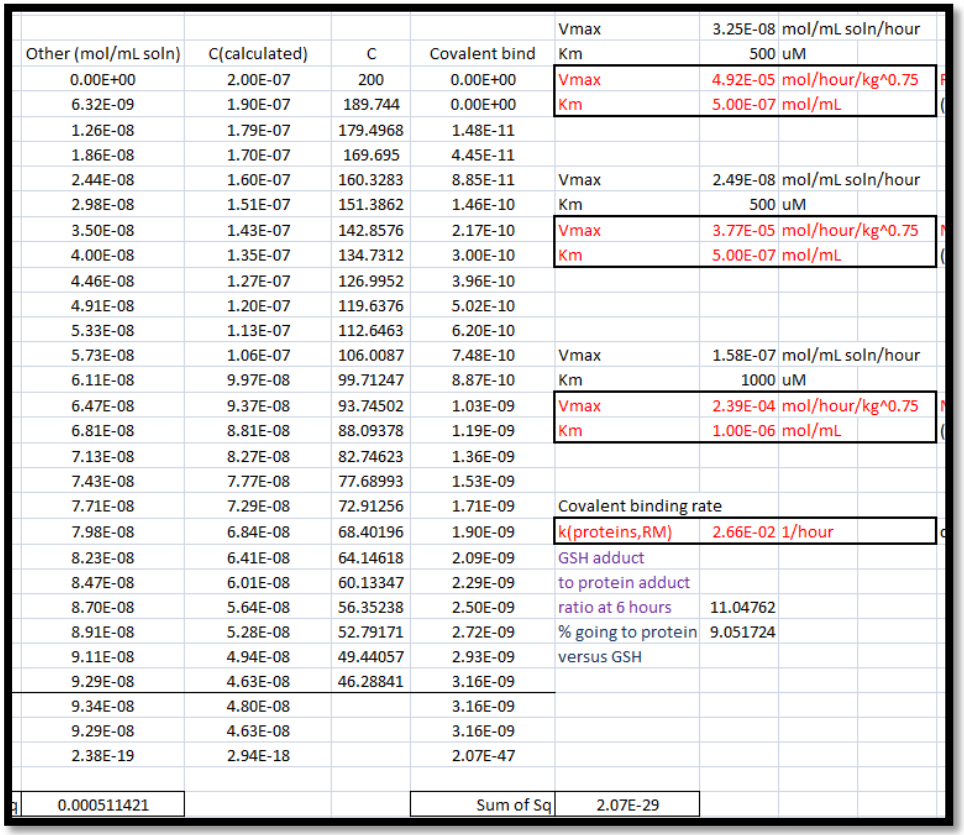




Ponsoda *Biochem Pharm* 1999

General Summary of Metabolism Data Used for RM Determination

- Microsomal metabolism data is key to the determination of reactive metabolite generation rates
  - RM and its products are often not easily measured *in vivo*
  - Also key to determining GSH binding rate, protein adduction rate, etc.
  - Training sessions contain detailed steps for converting microsomal metabolism data to V_{max} and K_m for input into DILIsym®
- *In vivo* data can be used to eliminate certain pathways
  - Furosemide example: no GSH depletion *in vivo* means that pathway can be ignored
- Mechanistic data are still required for determining active toxicity pathways
  - ROS is usually the appropriate pathway but not always



				Vmax	3.25E-08	mol/mL soln/hour
Other (mol/mL soln)	C(calculated)	C	Covalent bind	Km	500	uM
0.00E+00	2.00E-07	200	0.00E+00	Vmax	4.92E-05	mol/hour/kg^0.75
6.32E-09	1.90E-07	189.744	0.00E+00	Km	5.00E-07	mol/mL
1.26E-08	1.79E-07	179.4968	1.48E-11			
1.86E-08	1.70E-07	169.695	4.45E-11			
2.44E-08	1.60E-07	160.3283	8.85E-11	Vmax	2.49E-08	mol/mL soln/hour
2.98E-08	1.51E-07	151.3862	1.46E-10	Km	500	uM
3.50E-08	1.43E-07	142.8576	2.17E-10	Vmax	3.77E-05	mol/hour/kg^0.75
4.00E-08	1.35E-07	134.7312	3.00E-10	Km	5.00E-07	mol/mL
4.46E-08	1.27E-07	126.9952	3.96E-10			
4.91E-08	1.20E-07	119.6376	5.02E-10			
5.33E-08	1.13E-07	112.6463	6.20E-10			
5.73E-08	1.06E-07	106.0087	7.48E-10	Vmax	1.58E-07	mol/mL soln/hour
6.11E-08	9.97E-08	99.71247	8.87E-10	Km	1000	uM
6.47E-08	9.37E-08	93.74502	1.03E-09	Vmax	2.39E-04	mol/hour/kg^0.75
6.81E-08	8.81E-08	88.09378	1.19E-09	Km	1.00E-06	mol/mL
7.13E-08	8.27E-08	82.74623	1.36E-09			
7.43E-08	7.77E-08	77.68993	1.53E-09			
7.71E-08	7.29E-08	72.91256	1.71E-09	Covalent binding rate		
7.98E-08	6.84E-08	68.40196	1.90E-09	k(proteins,RM)	2.66E-02	1/hour
8.23E-08	6.41E-08	64.14618	2.09E-09	GSH adduct		
8.47E-08	6.01E-08	60.13347	2.29E-09	to protein adduct		
8.70E-08	5.64E-08	56.35238	2.50E-09	ratio at 6 hours	11.04762	
8.91E-08	5.28E-08	52.79171	2.72E-09	% going to protein	9.051724	
9.11E-08	4.94E-08	49.44057	2.93E-09	versus GSH		
9.29E-08	4.63E-08	46.28841	3.16E-09			
9.34E-08	4.80E-08		3.16E-09			
9.29E-08	4.63E-08		3.16E-09			
2.38E-19	2.94E-18		2.07E-47			
0.000511421			Sum of Sq	2.07E-29		