Development and Application of In Vitro Models for Screening Drugs and Environmental Chemicals that Predict Toxicity in Animals and Humans

By: Jim McKim, Ph.D., DABT
Founder and Chief Science Officer

CeeTox

Presentation Topics

- Introduction to CeeTox Laboratories
- Overview of services
- Description of our Systemic Toxicity Model
- Predicting an in vitro LD50

CeeTox ,Inc.: Is a Specialty Contract Research Laboratory Located in Kalamazoo Michigan at the Southwest Michigan Innovation Center

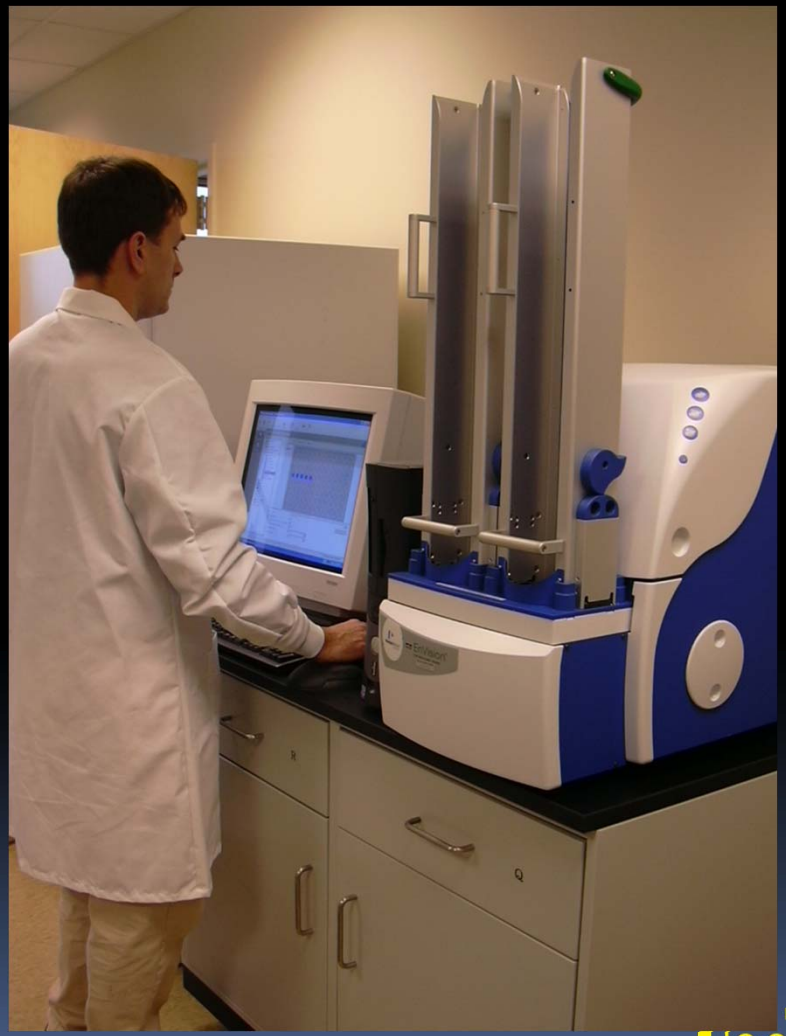
- Founded 2003
- Focus on in vitro toxicity
- Currently 32 employees
- Owned by NAMSA, Inc 2008

CeeTox Customer Base

Small and midsized Pharma
Personal care companies
Household product companies
Medical device
Petroleum companies
Government agencies

CeeTox has a World Class Cell Culture and In Vitro Toxicology Laboratory

CeeTox Utilizes Robotics and State-of-the- Art Laboratory Equipment to Process Samples



CeeTox Has a Wide Range of Assay Capabilities all of Which can be Done in a High Throughput Mode

- Roche 480 thermocycler 96- and 384-well head
- BioTek plate reader
- Perkin Elmer Fusion
- Roche xCelligence system for cardiac toxicity
- Beckman Coulter Biomek Robotics Station

CeeTox Intellectual Property

CeeTox Patents

New In Vitro Screening Method	Issued
CardioTox	Issued
Anti-Tumor Predictive Tox	Pending
Skin Sensitization	Pending
Multi-Organ Prediction	Pending

CeeTox Overview of Services

… a wide range of *in vitro* toxicology and safety assessment services

- **Systemic Toxicity Services**
  - CTOX Panel®
  - Acute Toxicity Screen
  - In Vitro LD50
- **Dermal Toxicity**
  - Percutaneous Absorption
  - Corrosion
  - Irritation
  - Sensitization
  - Photo Toxicity
- **Ocular Toxicity**
- **Endocrine Disruption**
  - Estrogen receptor (ER) binding
  - Estrogen transcriptional activation
  - Androgen receptor (AR) binding
  - Steroidogenesis
  - Aromatase
- **Organ Specific Toxicity Models**
  - Heart (CardioTox®)
  - Kidney
  - Liver
- **ADME Serviced**
  - **CYP Induction/Inhibition**
  - **Caco2 absorption**
  - **Pgp transport**
  - **Metabolism**
  - **Metabolic stability**
  - **BSEP inhibition**
- **Drug / Drug Interaction**
- **Target / Pathway Assays**
  - **Acute Inflammatory Response**
  - **Apoptosis**
  - **Cell Proliferation**
  - **Cell Viability**
  - **Glutathione Homeostasis**
  - **Hepatobiliary Toxicity**
  - **Lipidosis**
  - **Membrane Integrity**
  - **Metabolism**
  - **Mitochondrial Function**

Building *In Vitro* Models for Predicting *In Vivo* Toxicity

The Road From Pharmaceuticals to Environmental Chemicals

By: James McKim, Ph.D., DABT
Chief Science Officer

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What is Systemic Toxicity and What do We Want From Alternative Methods?

- **Toxicity that occurs after a chemical is absorbed into general circulation**
  - **Acute systemic toxicity**
    Single dose, and short exposure time
    Intrinsic toxicity of a chemical, **LD50**, organ effects
  - **Subacute systemic toxicity**
    Repeated-dose study, typically 14 day
    Information on toxicity following repeated exposure
    Helps establish doses for subchronic studies
  - **Subchronic systemic toxicity**
    Repeated-dose, typically 28 and 90 days
    Organ specific effects
    Establish NOAEL and LOAEL
    Regulatory implications FDA and EPA
  - **Chronic systemic toxicity**

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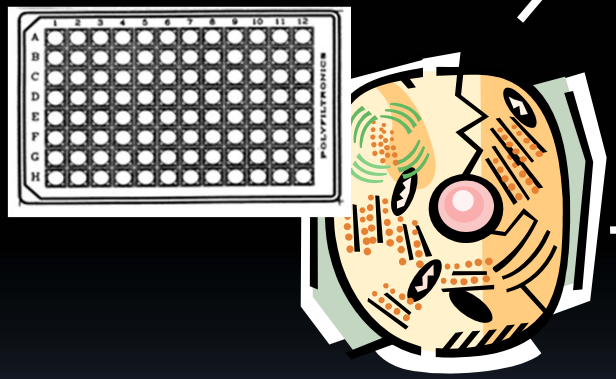
The Goal is to Predict Human Systemic Toxicity

Predicts Toxicity

Realistically



Ideally

What data are required for risk assessment
NOAEL, LOAEL
RfD, Benchmark dose

Predicts NOAEL

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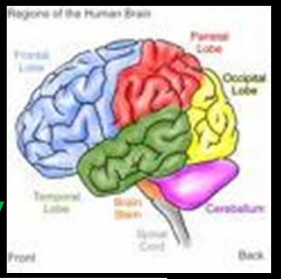
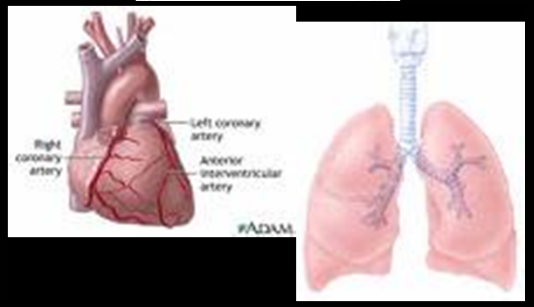
Building an In Vitro Model to Predict Repeat Dose Systemic Toxicity is Too Complex to Achieve Within the Next 10 Years!

Alternative approach is
to identify a plasma concentration
that results in general systemic toxicity

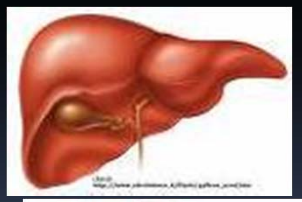


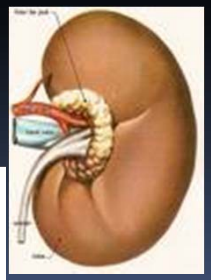


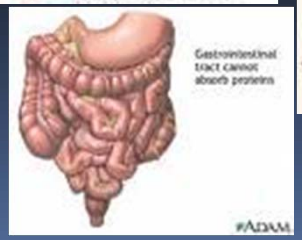
Most models
focus on
predicting
organ specific
toxicity

BARRIER to SUCCESS

Focus on the type of data needed
to make decisions







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In Vitro Models Should Provide Data That Can be Used to Make Decisions

What data are required from an in vitro method in order to make decisions on systemic toxicity?

- Minimum dose that results in toxicity
- Maximally tolerated dose
- Plasma concentration that results in toxicity
- LOAEL
- NOAEL
- Margin of Safety

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Defining a Working Hypothesis

If the in vivo (animal) plasma concentration versus time and toxicity curves can be related to In vitro concentration versus toxicity response curves for a large number of compounds, then a relationship between the animal response data and the in vitro response data can be explained mathematically.

Relating Plasma Concentration to Toxicity

- **Start with pharmaceuticals**
  - Intended for internal exposure at higher doses for shorter time
  - Environmental chemicals typically not intended to be ingested, exposure to lower doses, but for longer times
- **Develop a means to estimate the plasma concentration where general toxicity would occur**
  - Develop a relationship between concentration response curves *in vitro* and Cmax *in vivo* at the dose where toxicity was observed in rat 14-day repeated oral dose studies
    - Clinical chemistry
    - Histopathology

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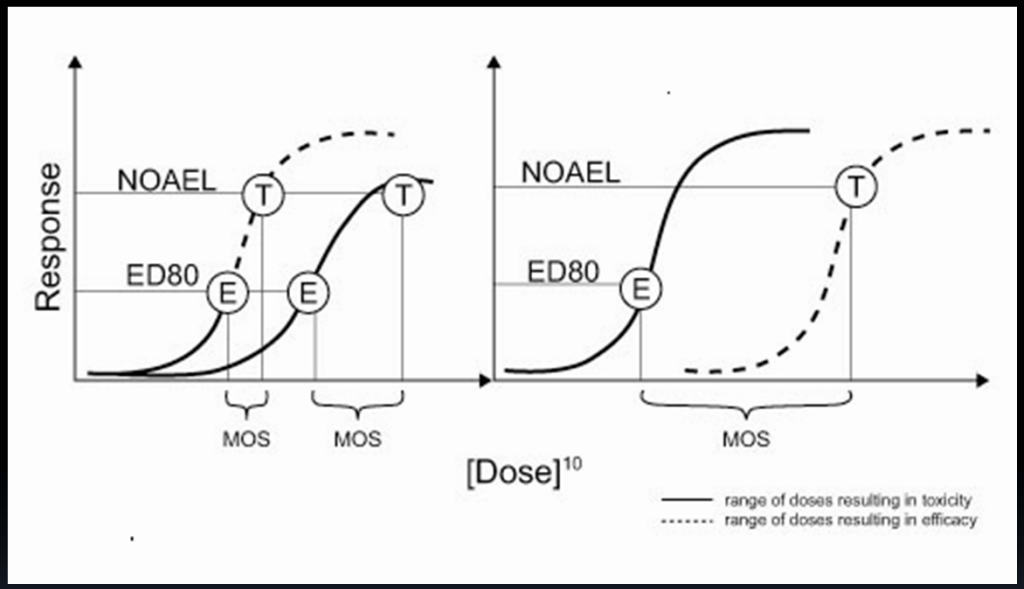
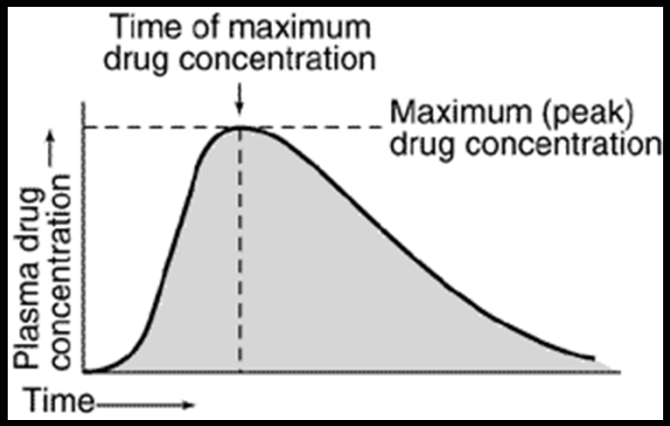
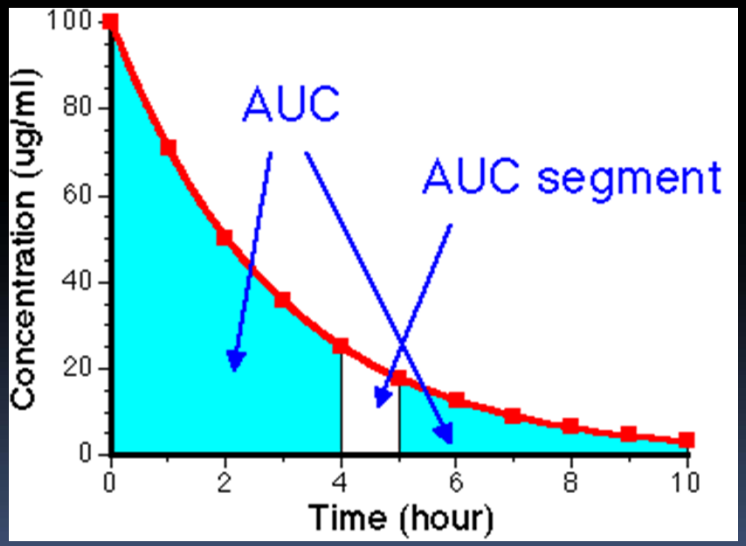
The Relationship Between In Vivo Plasma Concentration (Bioavailability) and Toxicity Provides the Basic Model

In vivo and In vitro data



JL Stevens (2006) Chem Res Toxicol 19, 1393-1401





Toxicity increases with potency = target
Cell models that lack target show toxicity = chemistry

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Building the Predictive Model Based on Empirical Data Sets

- Acquired in vivo data for pharmacokinetic parameters
- Developed concentration response data in vitro
- Based on multiple endpoint analysis developed a
- relationship between in vivo and in vitro data sets
- Test the model with small number of drugs
- Validate model in large blinded study
- Evaluate relevance of method for determining effects in humans

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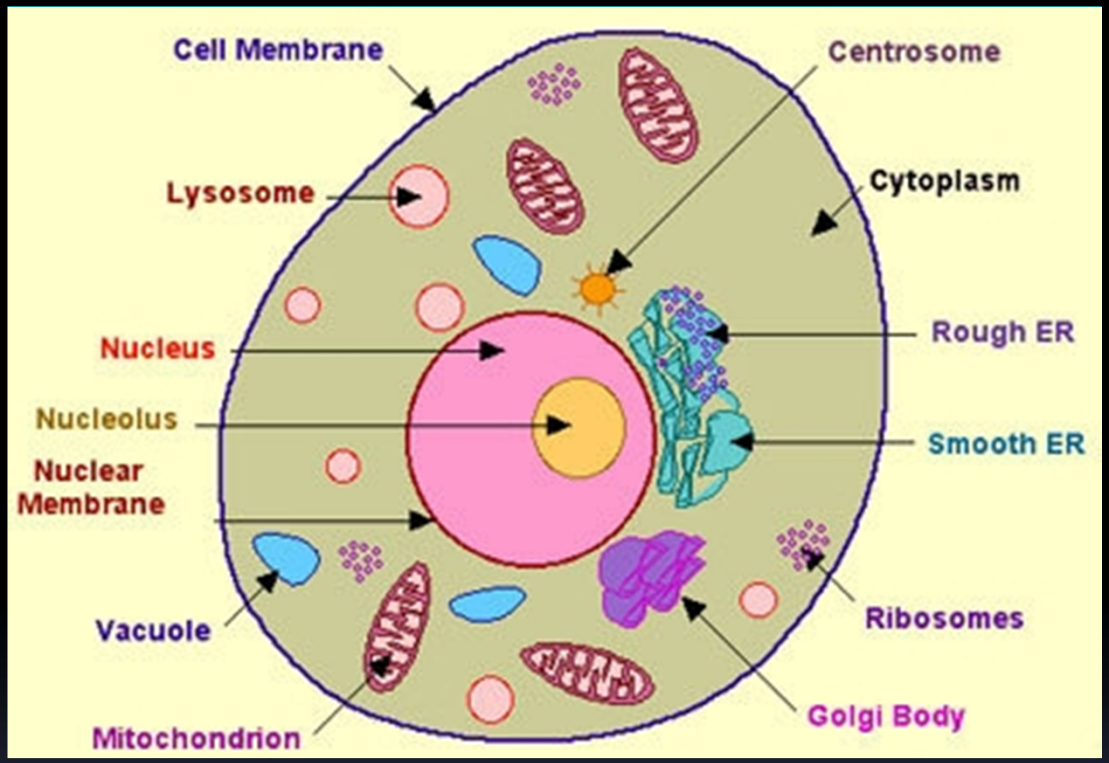
Focus on Cell Biology and Physical Chemical Properties Improves the Model



Biochemical Function

Membrane Integrity
Mitochondrial Function
Cell Proliferation
Redox-State
Oxidative Stress
Apoptosis

Physical-Chemical Properties

Solubility
Partition coefficients
Pka
Protein binding
Metabolic stability
Metabolic activation
Transporter interaction

Pharmacology

CNS receptors
Cardiovascular receptors and ion channels

Pharmacokinetic Parameters

Clearance
Bioavailability
Volume of distribution

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Pharmacokinetic Data are Essential for Building In Vitro Models That Have In Vivo Relevance

- Metabolic stability
- Clearance
- Absorption
- Protein binding (Kd = on vs off kinetics)
- Metabolic activation
- Bioavailability
- Volume of distribution

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Selecting a Cell Model That Consistently Provides Accurate Data

- **Rat hepatoma (H4IIe) Cell line**
  - Retains oxidative metabolism
  - Low constitutive metabolism
  - Accepts a wide range of serum concentrations

- **Human hepatocyte model (HepaRG)**

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Determining the Relationship Between *In Vitro* Concentration-Response and *In Vivo* Plasma Levels

- **Multiple endpoints improve interpretation**
  - Membrane integrity
  - Cell number or mass
  - Mitochondrial function
- **Analysis and comparison to *in vivo* plasma $C_{max/AUC}$**
- **Development of weighting factors for each endpoint**
- **Result was an estimate of the plasma concentration where toxicity would be expected to occur (C_{tox})**

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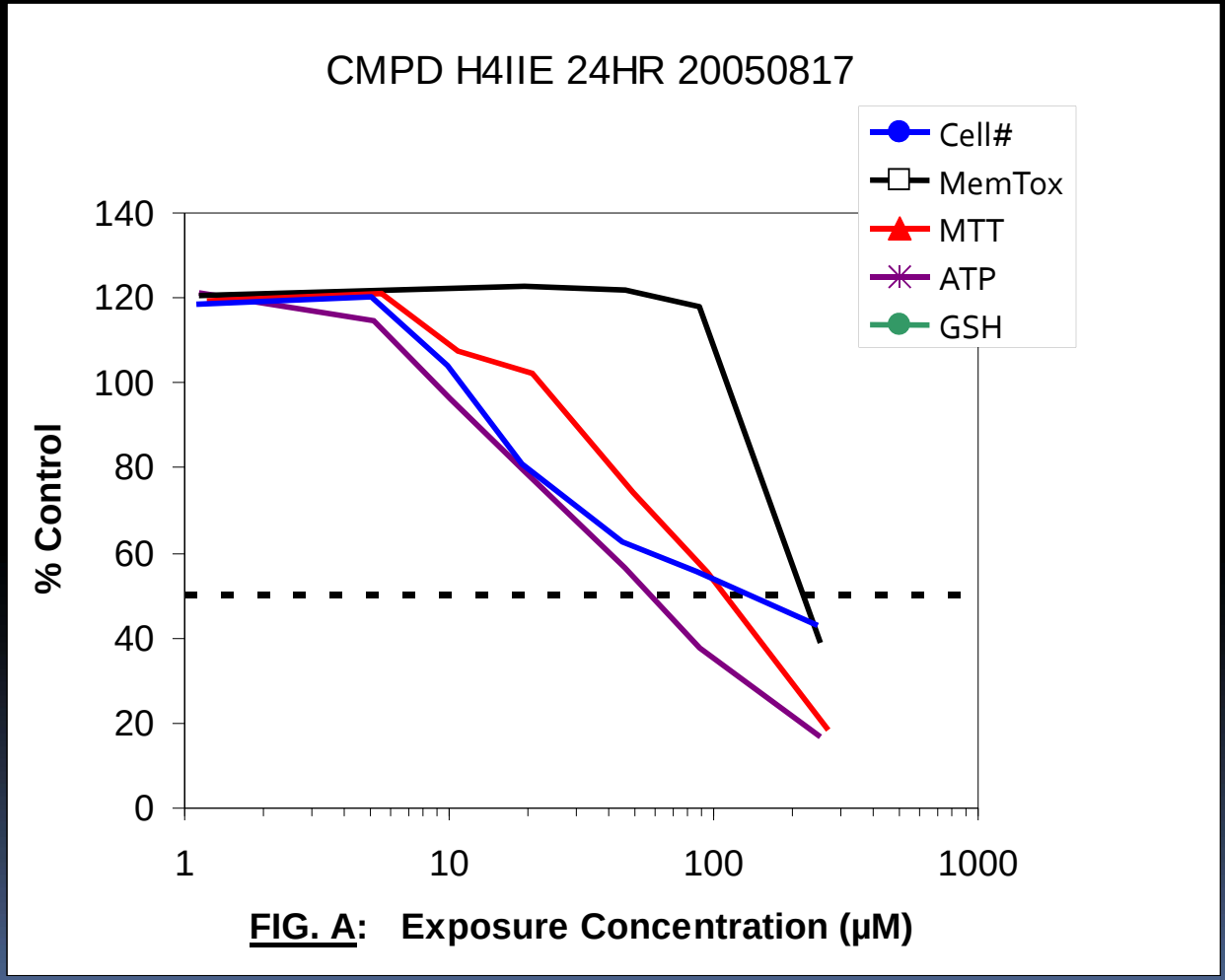
Multiple Endpoints Are Essential For Correct Interpretation of In Vitro Data



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Important Take Home Points

- Multiple endpoints based on cellular function are important
- Understanding the cell model
- Knowing what each assay is actually measuring

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Determining Weighting Factors

- **Membrane integrity**
  - Highest weight = cell death
  - Concentration response and potency
  - Time adjustment
- **Mitochondrial function**
  - Moderate weighting
  - Concentration response and potency
  - Time adjustment

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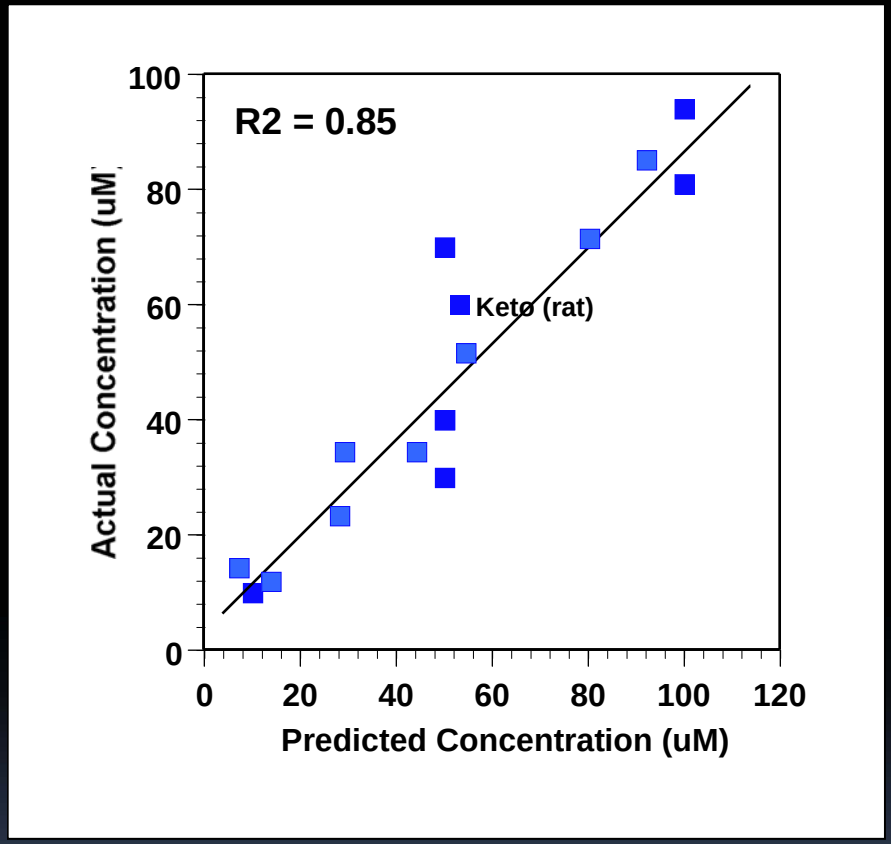
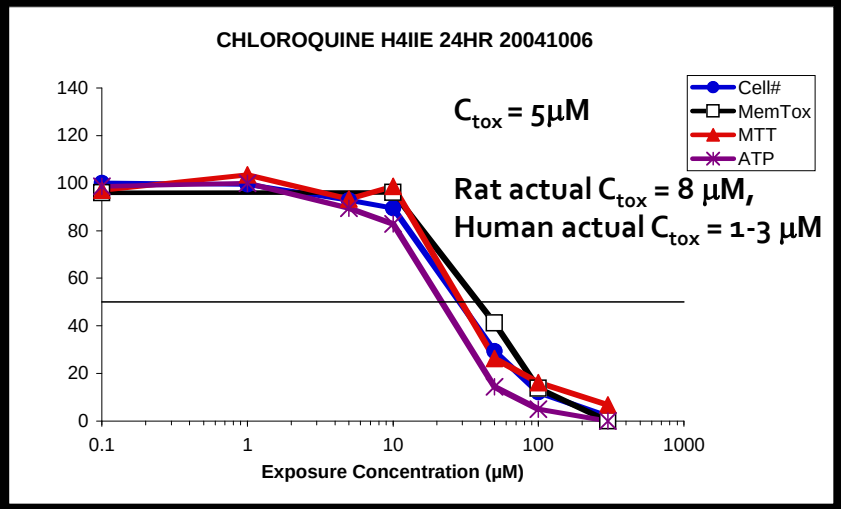
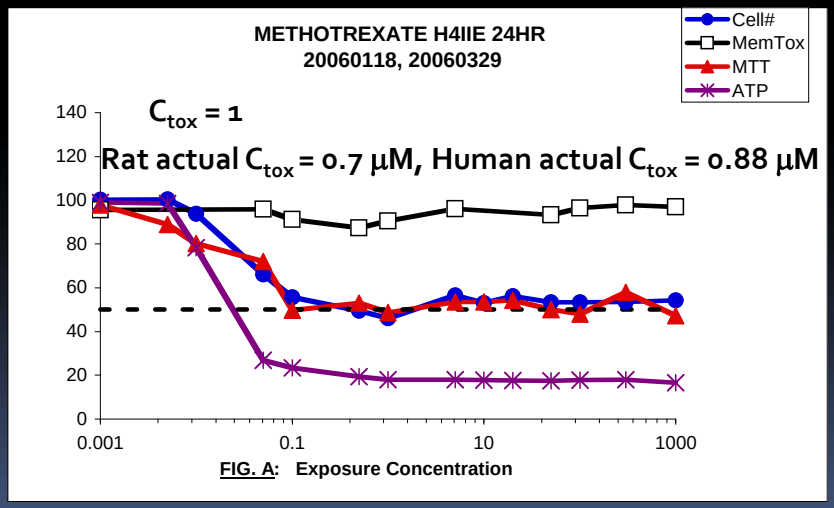
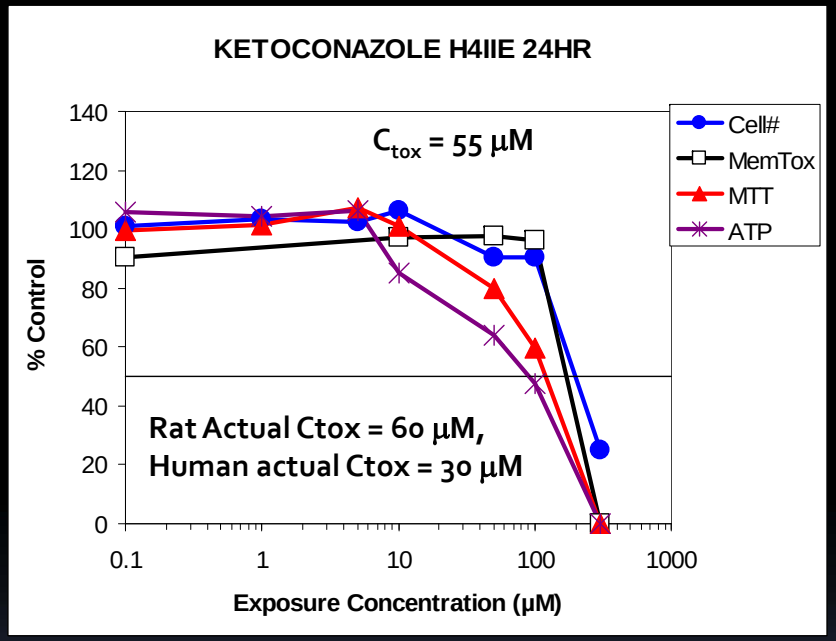
Evaluating Assumptions Against Rat *In Vivo* Data



In Vitro Toxicity Data Correlates With Adverse Effects in Animals and Humans







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Velcade is an Approved Drug With High In Vitro Toxicity?

Low Concentration
24 hr Exposure

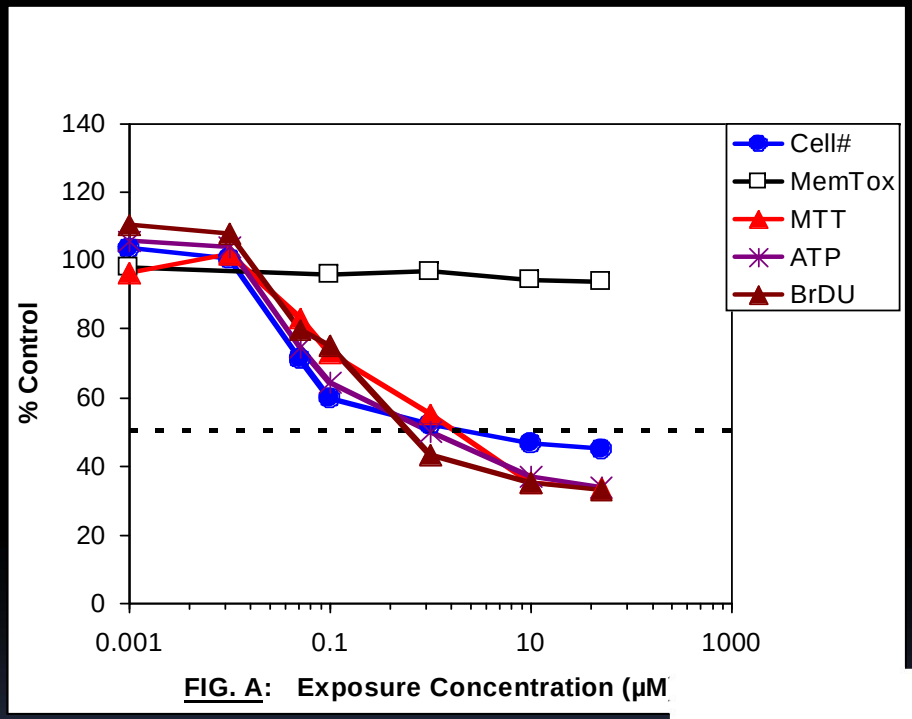
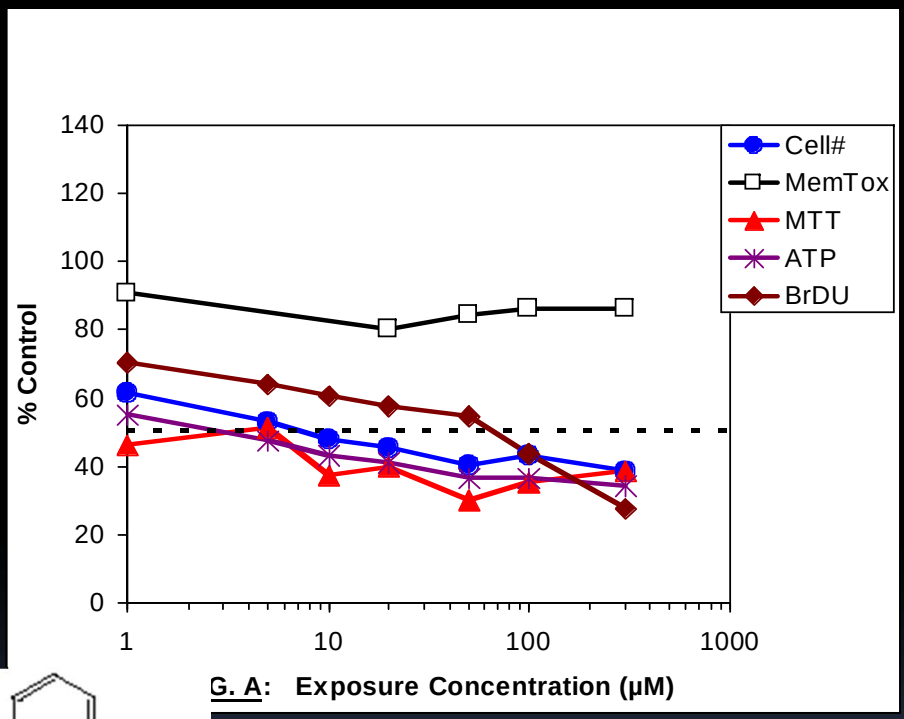
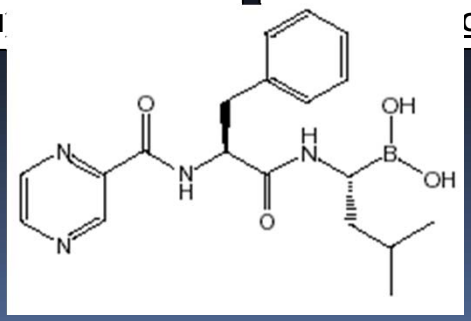


FIG. A: Exposure Concentration (µM)

High Concentration
24 hr Exposure



G. A: Exposure Concentration (µM)



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In Rat Primary (non-dividing) Hepatocytes Velcade Has Low Toxicity

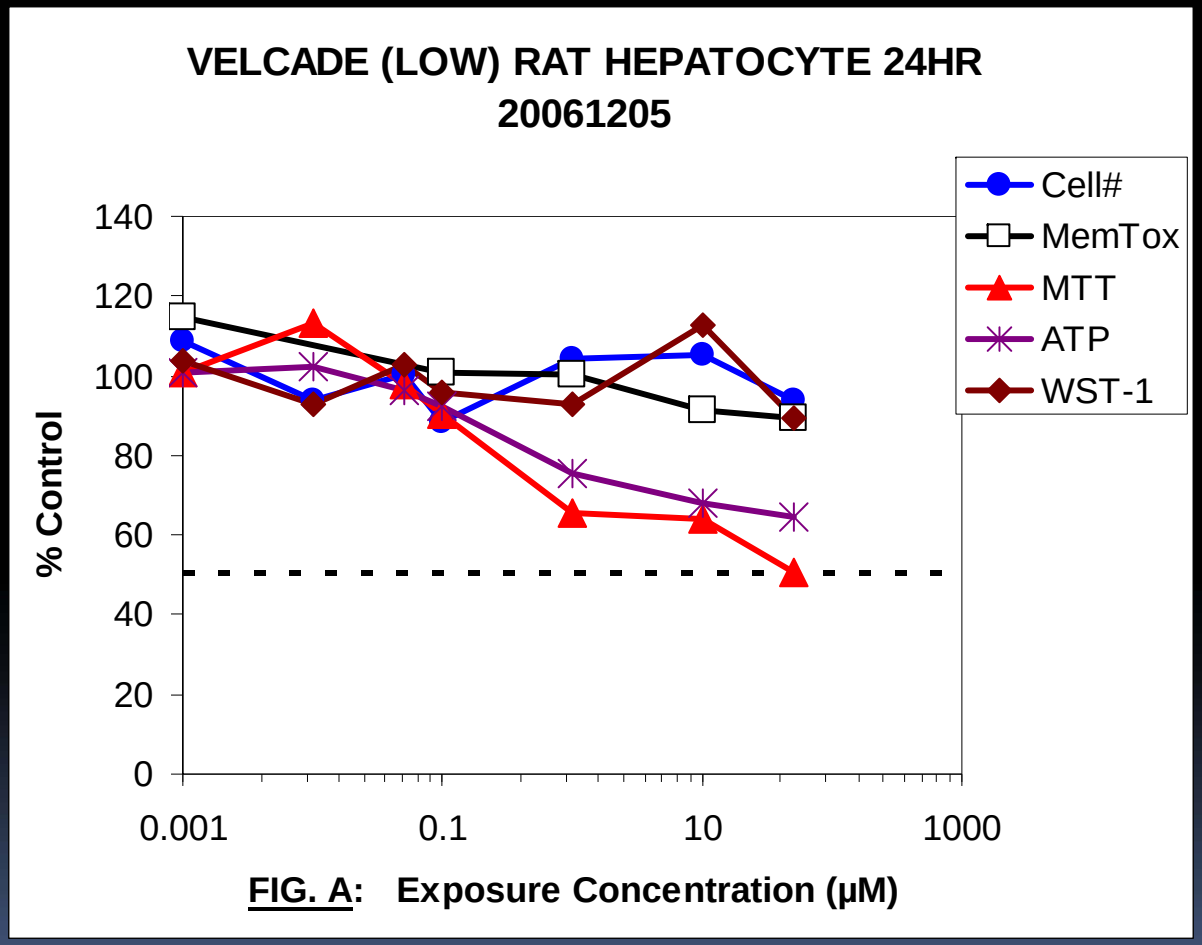
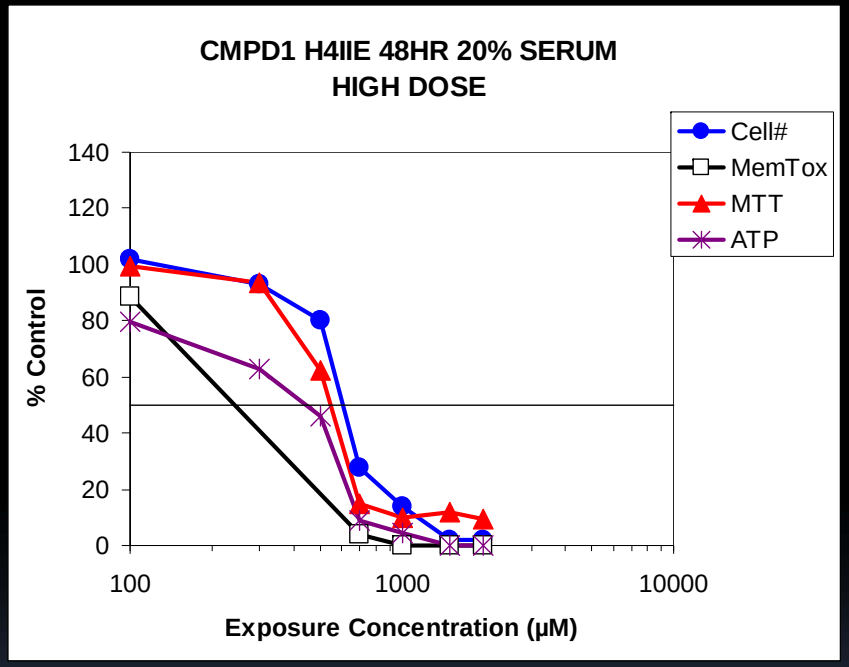
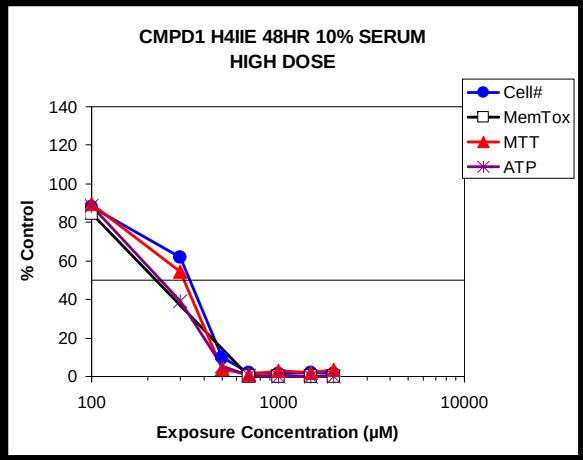
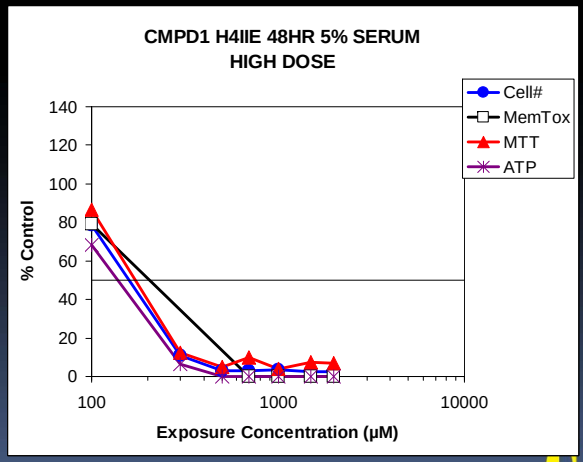


FIG. A: Exposure Concentration (µM)

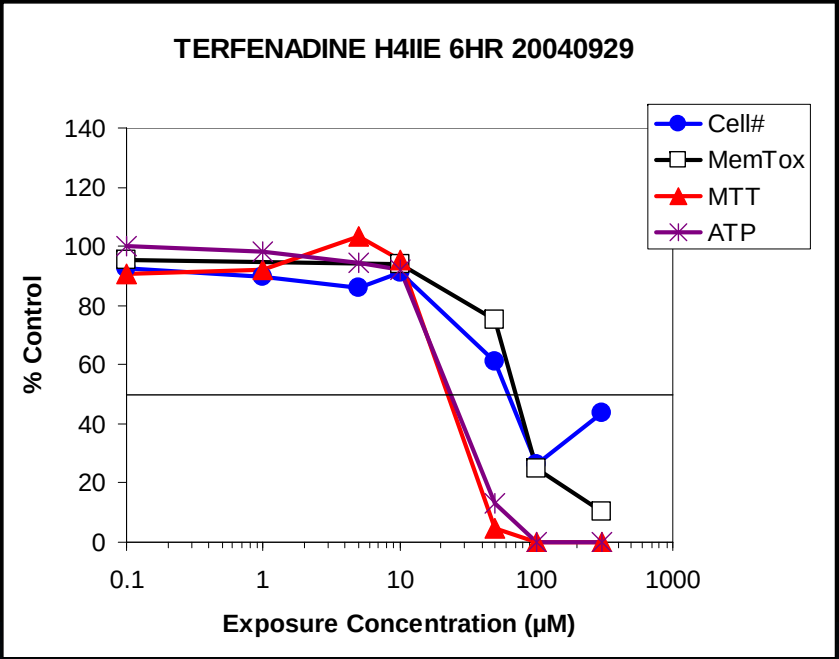
Protein Binding Affinity Can Impact Toxicity

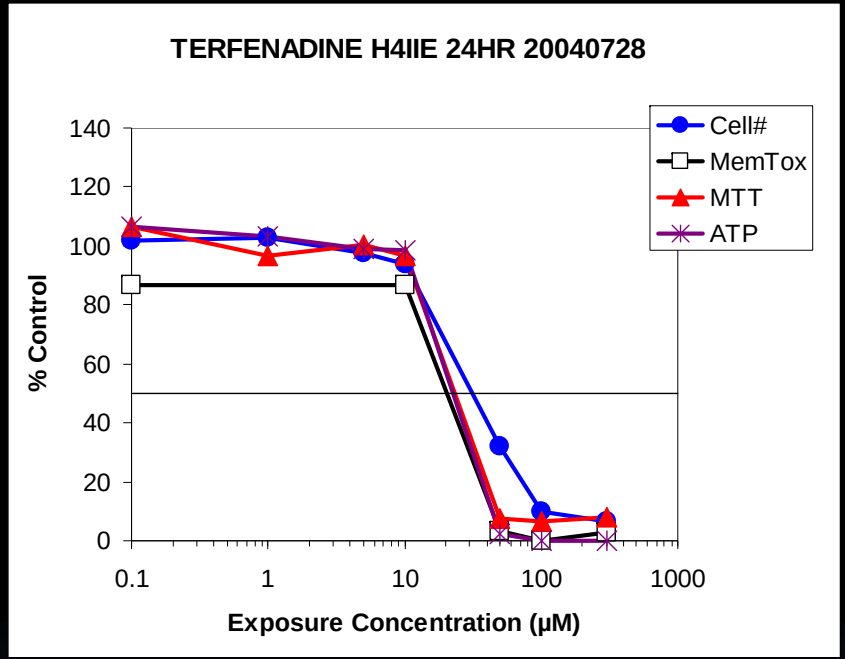






Metabolic Stability is Key for Correct Interpretation of In Vitro Data

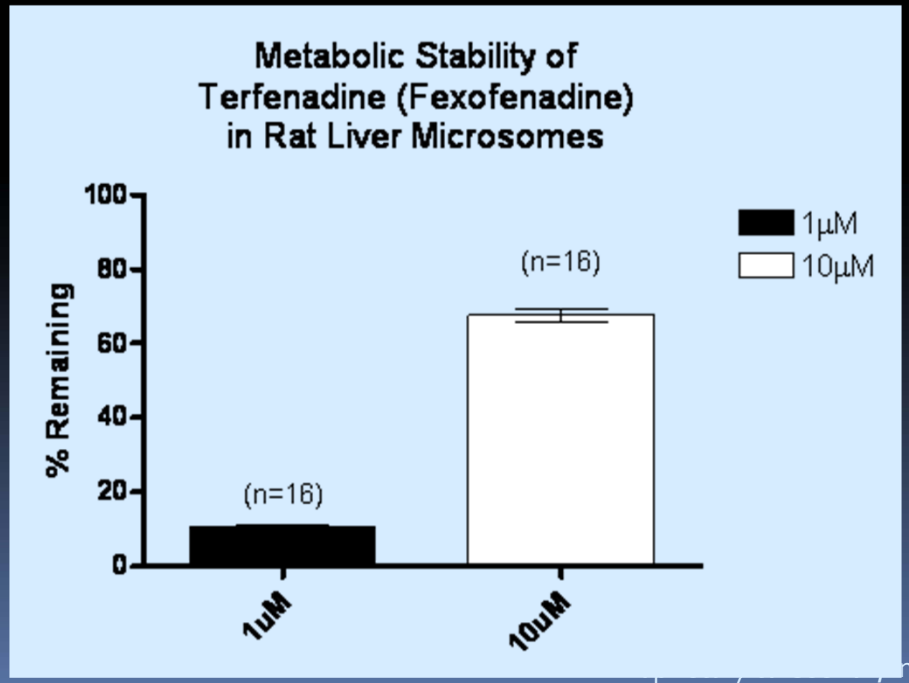




Considered safe in IND enabling safety studies
Considered safe in clinical trials
Why is it toxic *in vitro*?

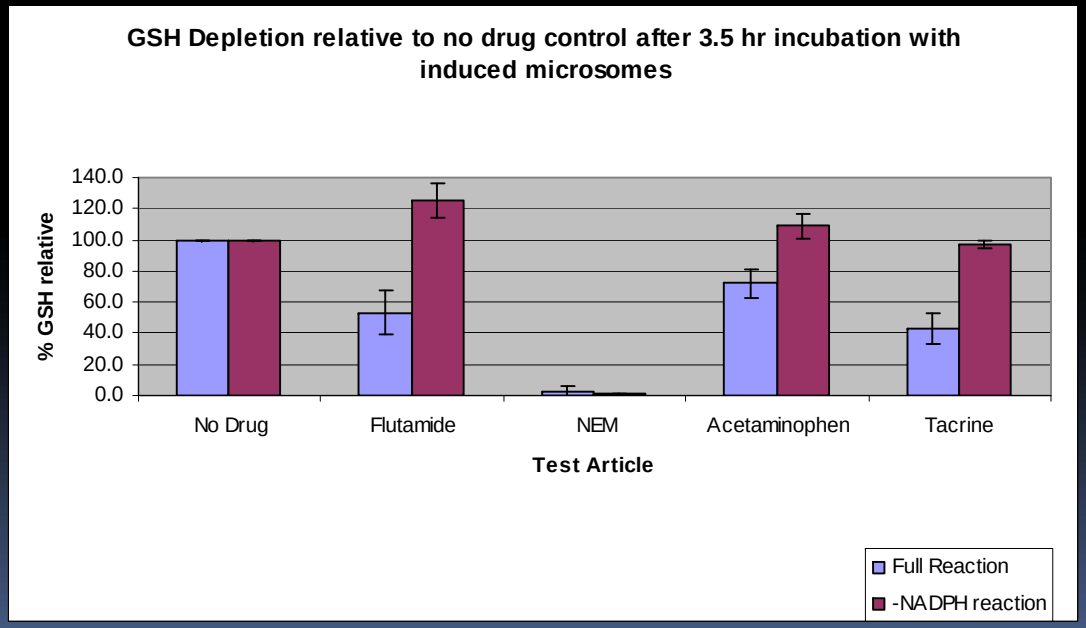
Importance of Metabolic Stability for Identifying False Positives

- *Terfenadine is a prodrug*
- Terfenadine undergoes first-pass metabolism
- Primary metabolite is efficacious not toxic
- *In Vivo* blood levels of terfenadine are low

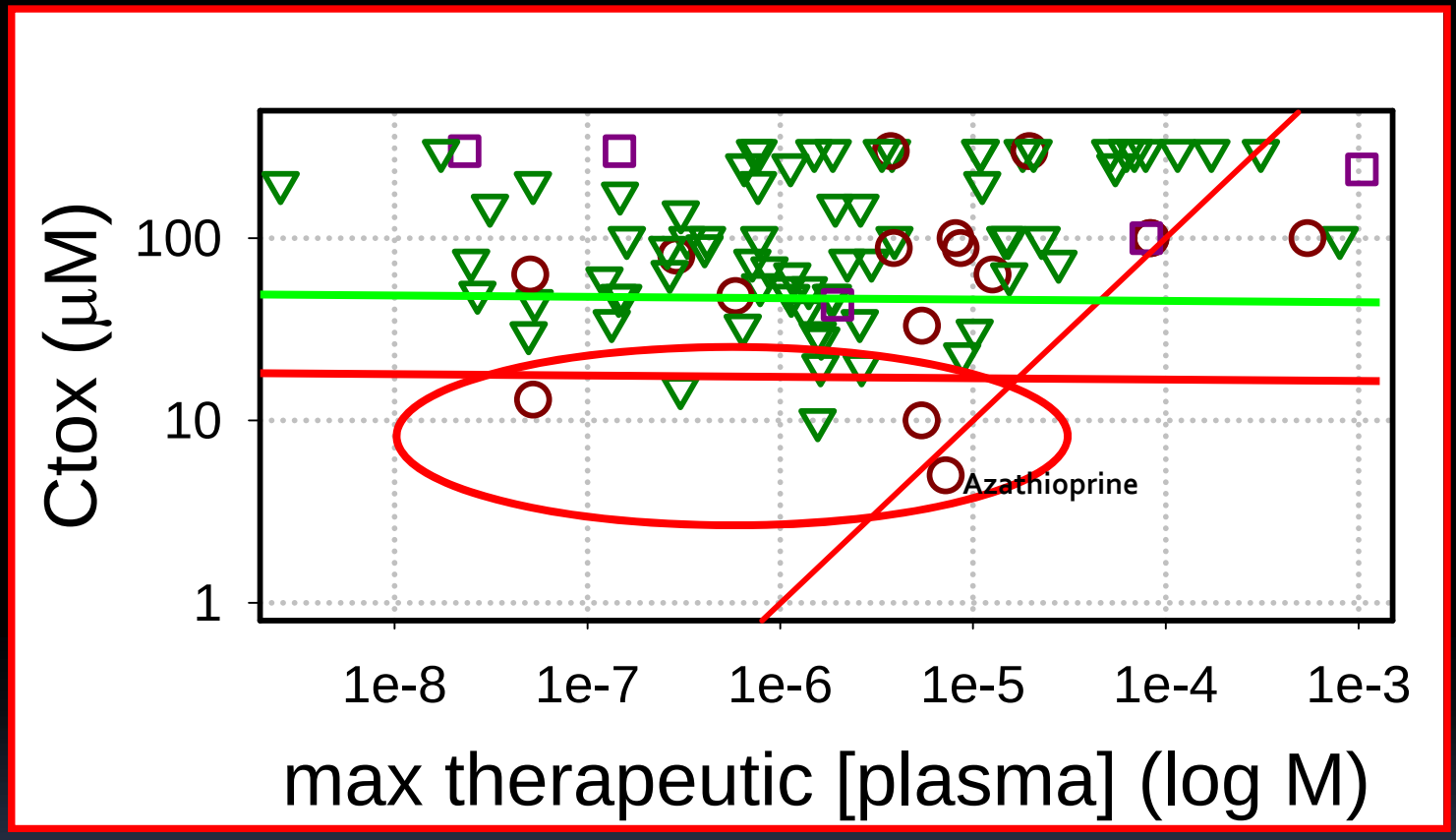


Metabolic Activation Identifies False Negative Results

- Tacrine = liver
- Flutamide = liver
- Acetaminophen = liver



Determining Relevance to Human Systemic Toxicity

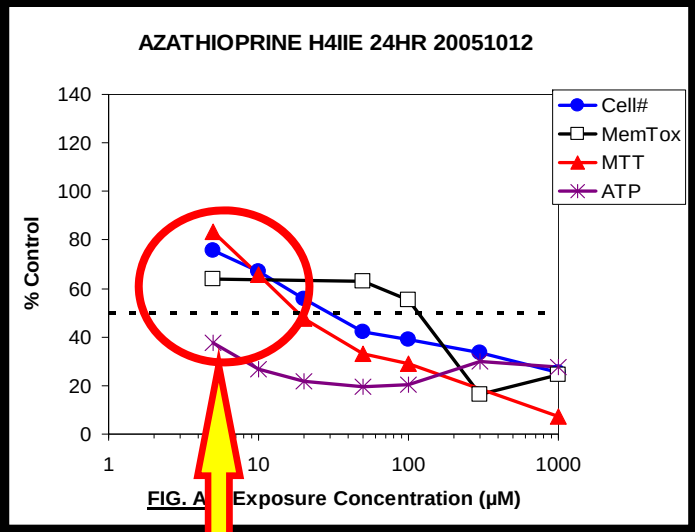


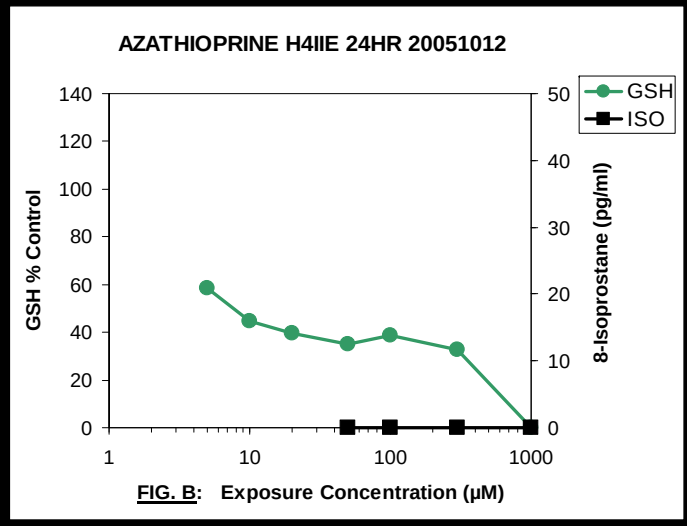
Ctox = 5 µM

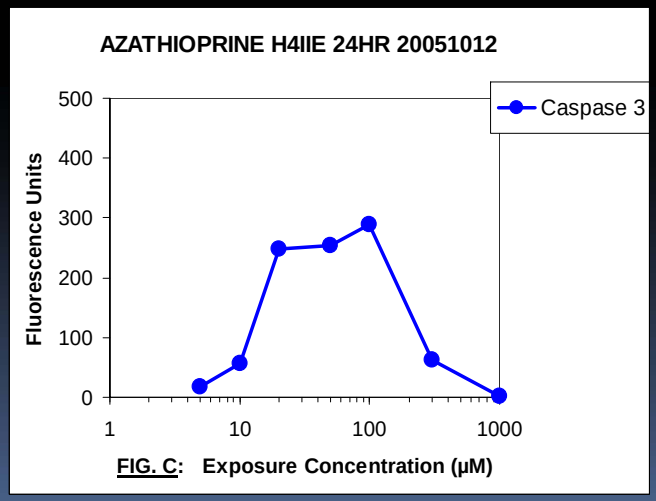
MTPC = 8 µM In vitro margin of safety <= 1

McKim, J. M., Jr. "Building a tiered approach to in vitro predictive toxicity screening: a focus on assays with in vivo relevance." Comb Chem High Throughput Screen 13(2): 188-206.

Azathioprine: Inhibits Purine Synthesis and Produces Toxicity Under Clinical Conditions







Extremely Toxic

C_{tox} = < 5 µM

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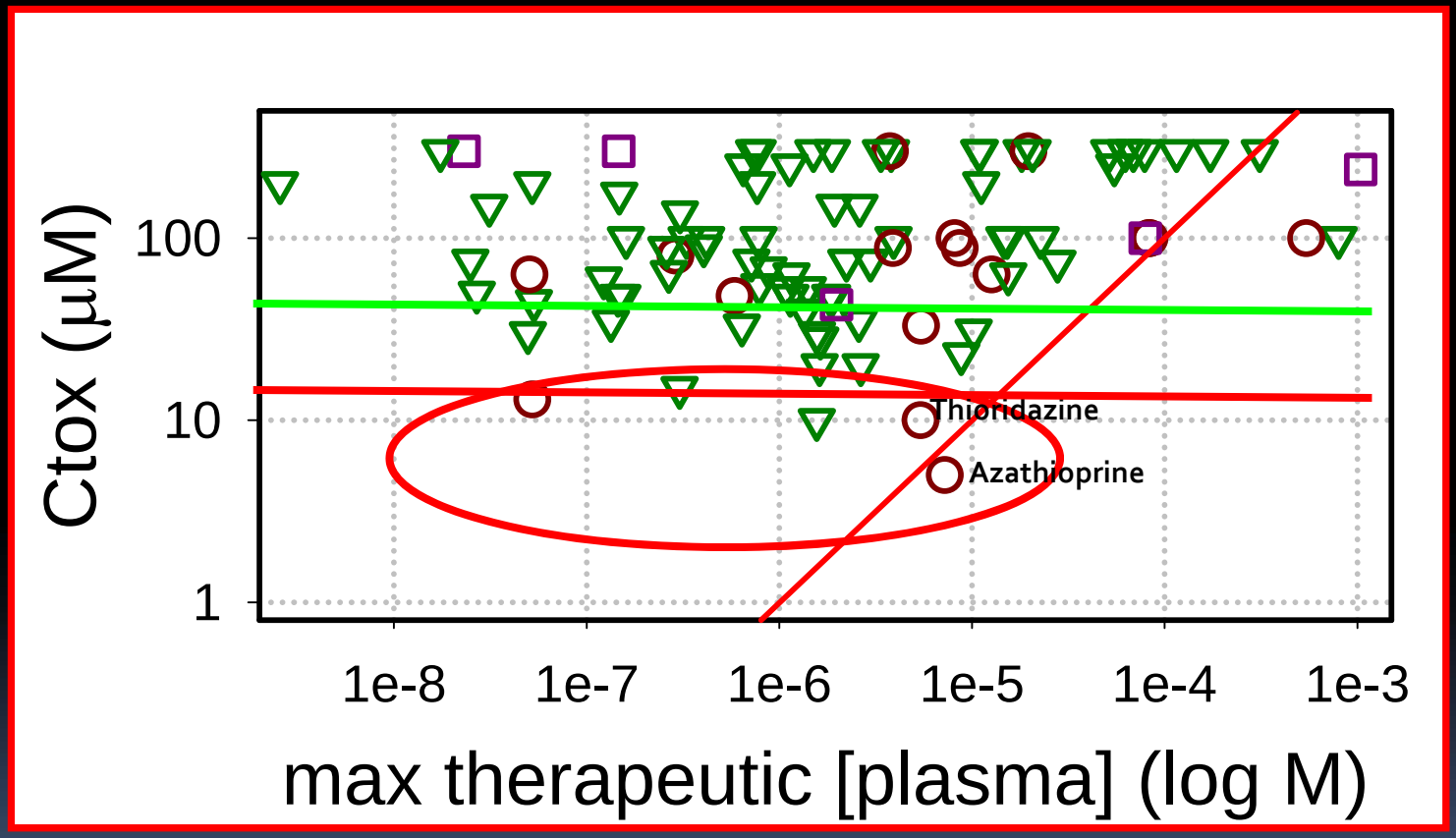
There is Good Concordance Between Predicted Toxicity Validated to Rat Data and Human Clinical Data



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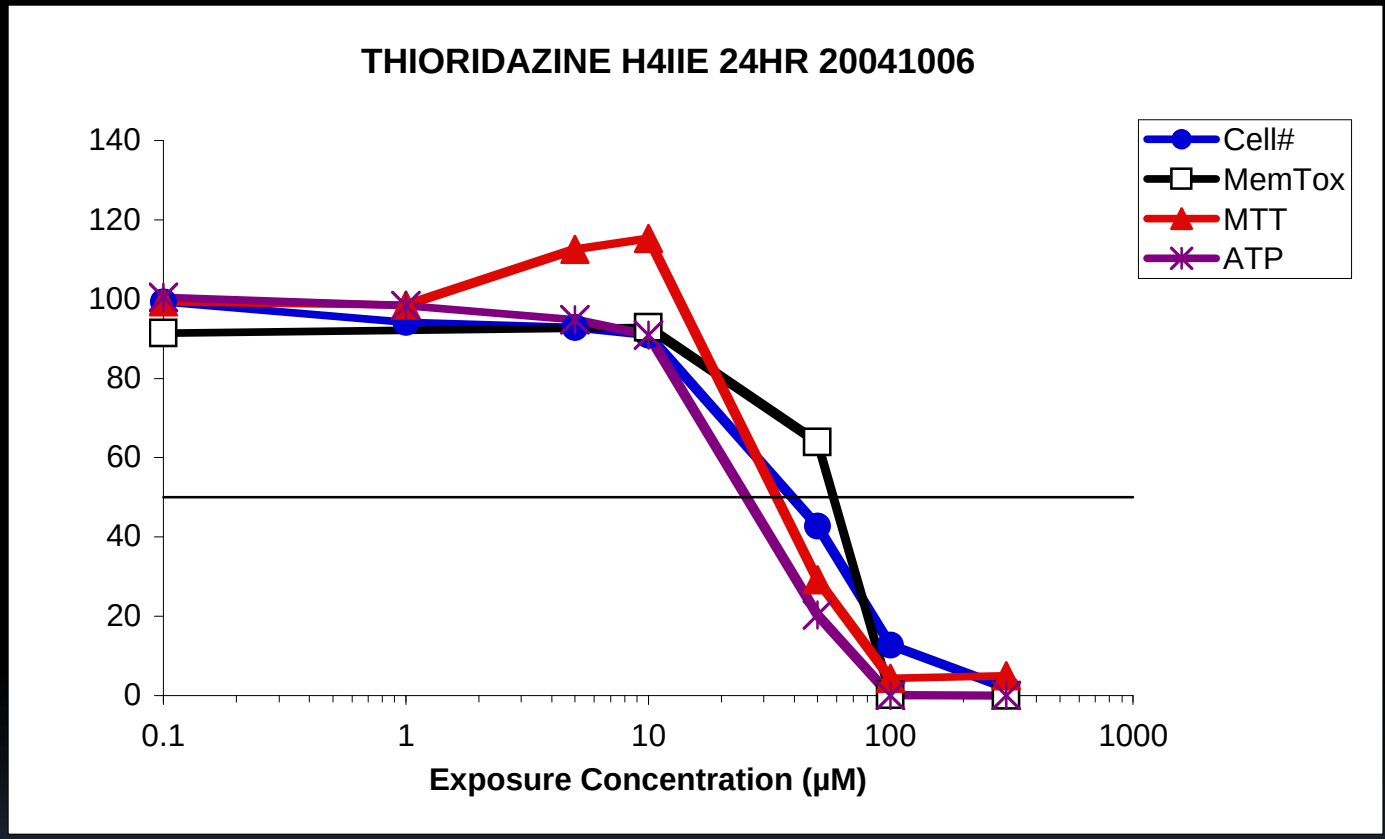

Ctox = 11 µM

MTPC = 6 µM In vitro margin of safety = 1.83

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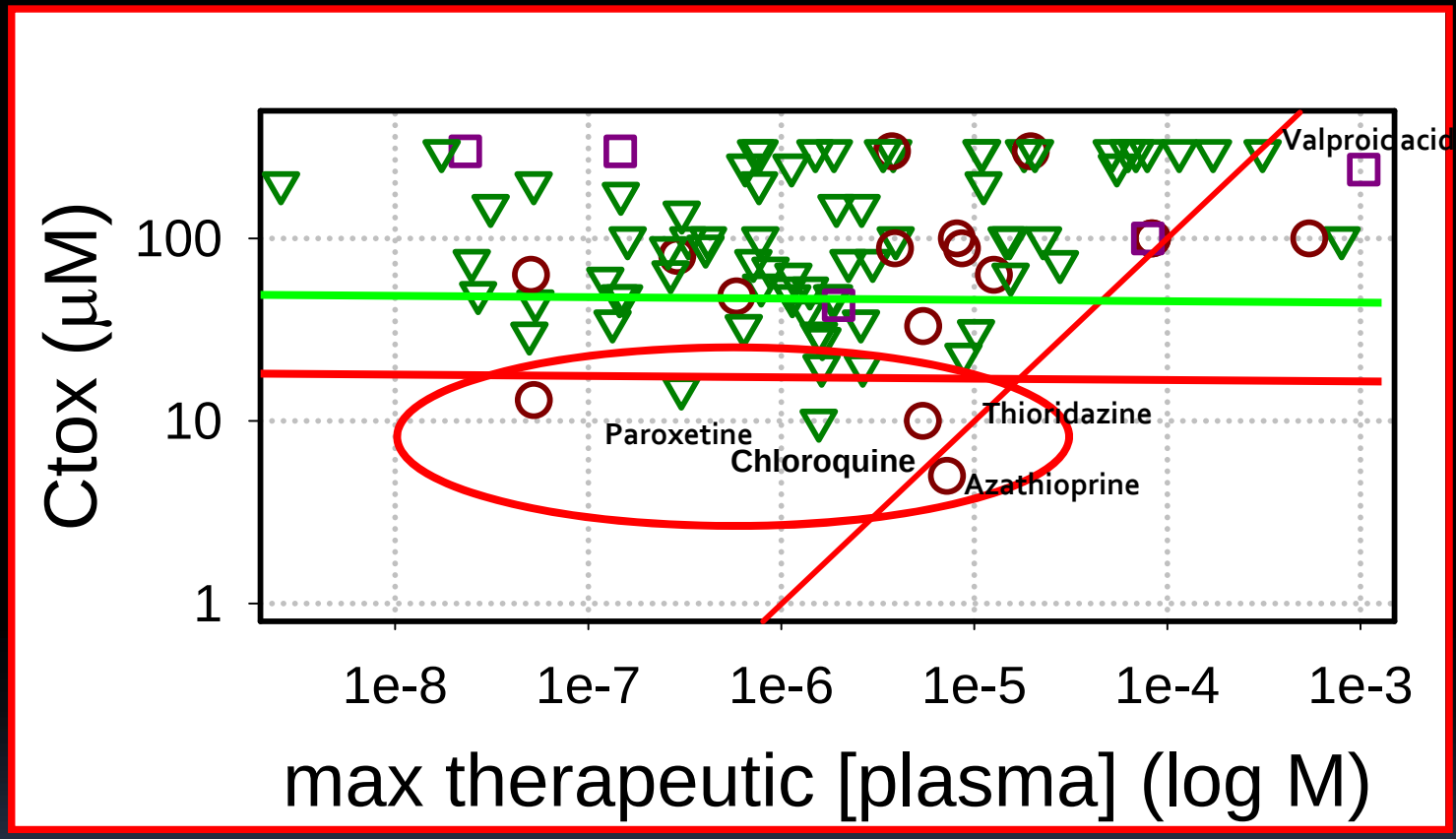
Drug Potency Can Influence Plasma Concentration and Toxicity



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Antidepressant Paxil (SSRI) Considered Safe Under Appropriate Use: Rare Incidences of Hepatotoxicity

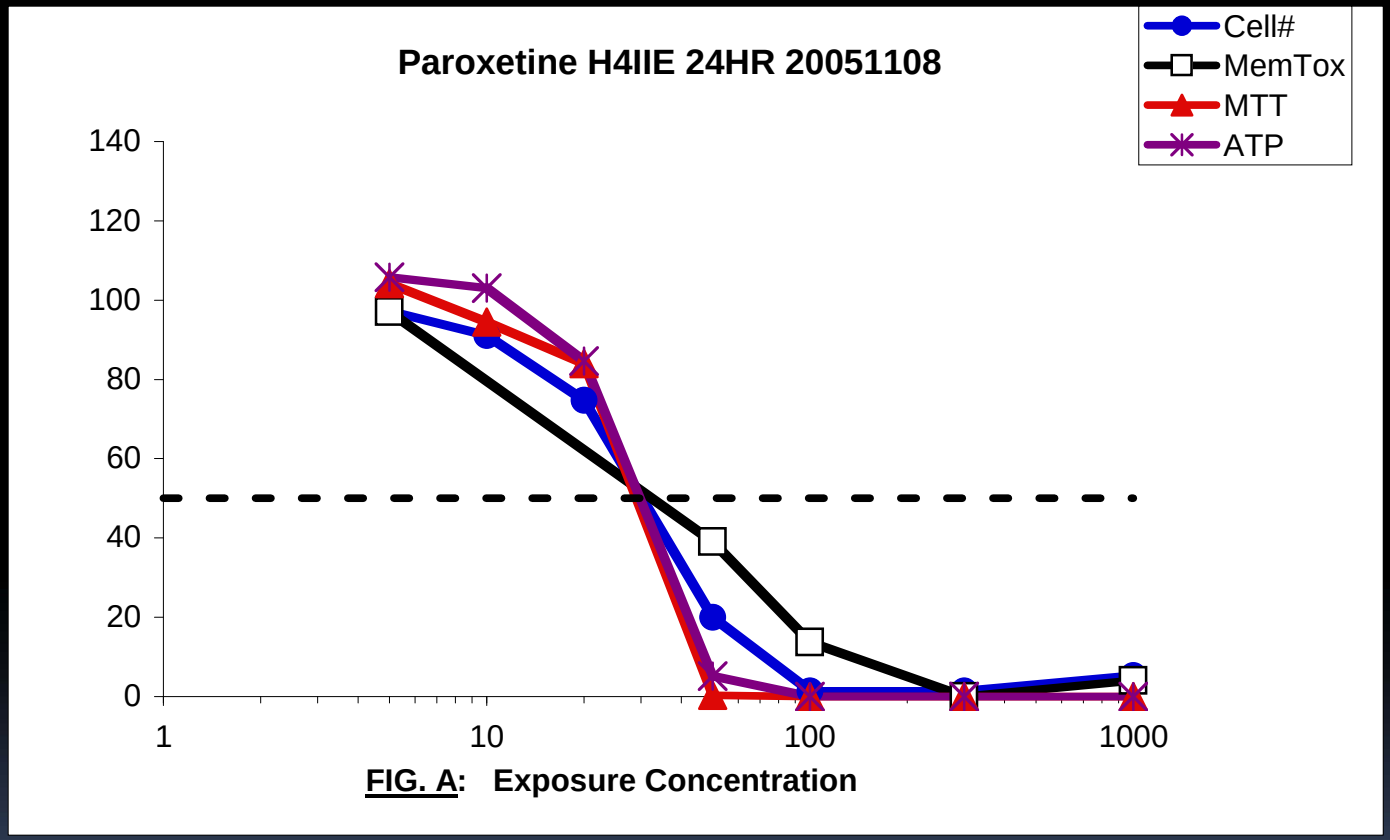

FIG. A: Exposure Concentration

Ctox = 15 µM

MTPC = 0.3 µM In Vitro Margin of Safety = 50

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Predicting Bioavailability of a New Drug and Maximum Therapeutic Plasma Concentration

- In Vitro CL_{int} (human microsomes)
- Scaled in vitro CL_{int}
- Calculate bioavailability (%f)
  - $f = 1 - E$
  - $E = f_u \times CL_{int} / Q_H$

Kuhnz and Gieschen (1998) Drug Metab Disp 26, 1120.

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What is Systemic Toxicity and What do We Want From Alternative Methods?

- **Toxicity that occurs after a chemical is absorbed into general circulation**
  - **Acute systemic toxicity**
    - Single dose, and short exposure time
    - Intrinsic toxicity of a chemical, **LD50**, organ effects
  - **Subacute systemic toxicity**
    - Repeated-dose study, typically 14 day
    - Information on toxicity following repeated exposure
    - Helps establish doses for subchronic studies
  - **Subchronic systemic toxicity**
    - Repeated-dose, typically 28 and 90 days
    - Organ specific effects
    - Establish NOAEL and LOAEL
    - Regulatory implications FDA and EPA
  - **Chronic systemic toxicity**

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Predicting a Rat Acute Oral LD50 Dose

- **Collaborative research effort with LOREAL Paris**
- **Integrative or systems biology + Computational Toxicology**
  - Multiple endpoints related to cell health
  - Receptor binding data (pharmacology)
  - Physical Chemical parameters
  - Approach has been evaluated with more than 200 chemicals
  - Posters at the World Congress in Rome 2009, SOT 2010
- **Results show that an integrated *in vitro* approach can provide good estimates of an LD50**

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Global Harmonization System for Acute Toxicity

Standard Categories

Acute Toxicity	Cat. 1	Cat. 2	Cat. 3	Cat. 4	Cat. 5
Oral (mg/Kg)	≤ 5	>5 ≤ 50	> 50 ≤ 300	> 300 ≤ 2000	Safe >2000

Revised Categories

Acute Toxicity	Cat. 1-2-3	Cat. 4	Cat. 5
Oral (mg/Kg)	≤ 300	>300 ≤ 500	> 2000

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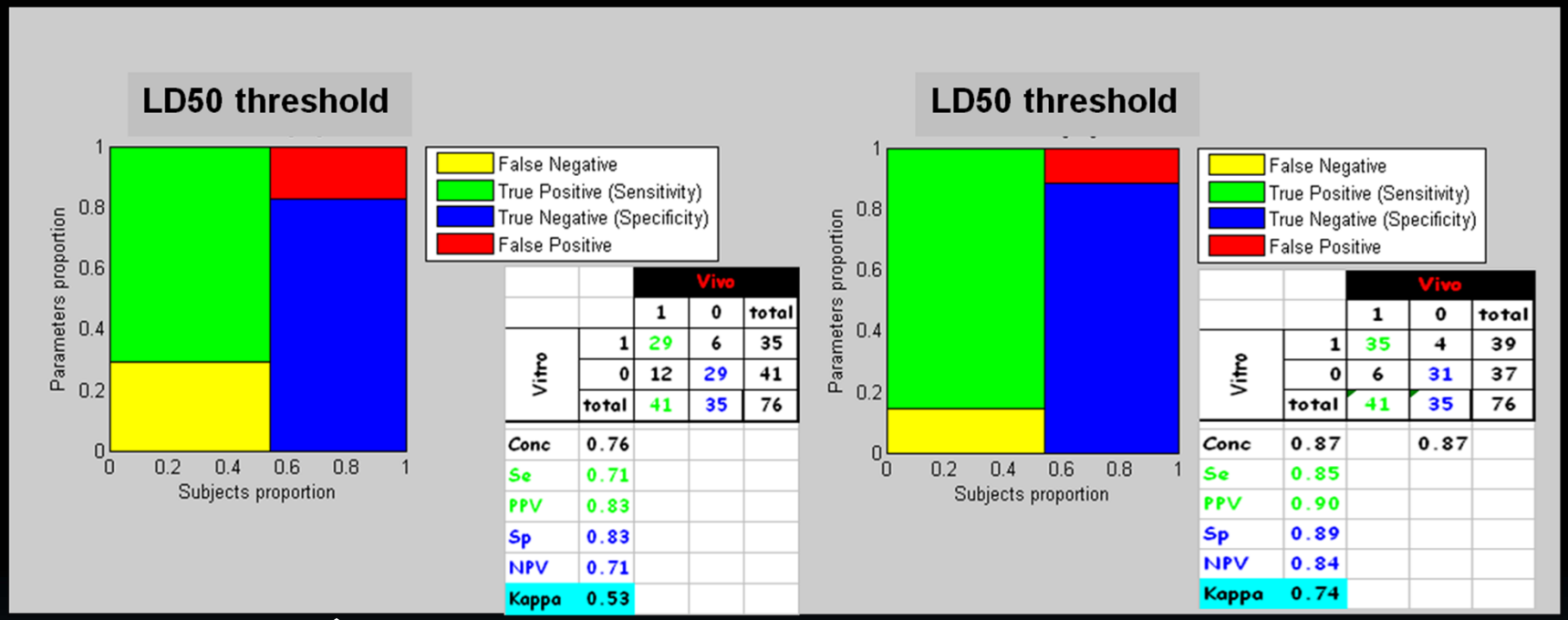
Addition of Pharmacology and Physical Chemistry Properties Improves Model Performance



Performance of the standard model at the LD50 threshold of 500 mg/kg

Performance of the integrated model at the LD50 threshold of 500 mg/kg

REDUCTION OF THE FALSE NEGATIVE RATE (yellow area)

Note, R et al. 2010 Presented at the World Congress on Alternatives to Animal Testing Montréal, August 2011

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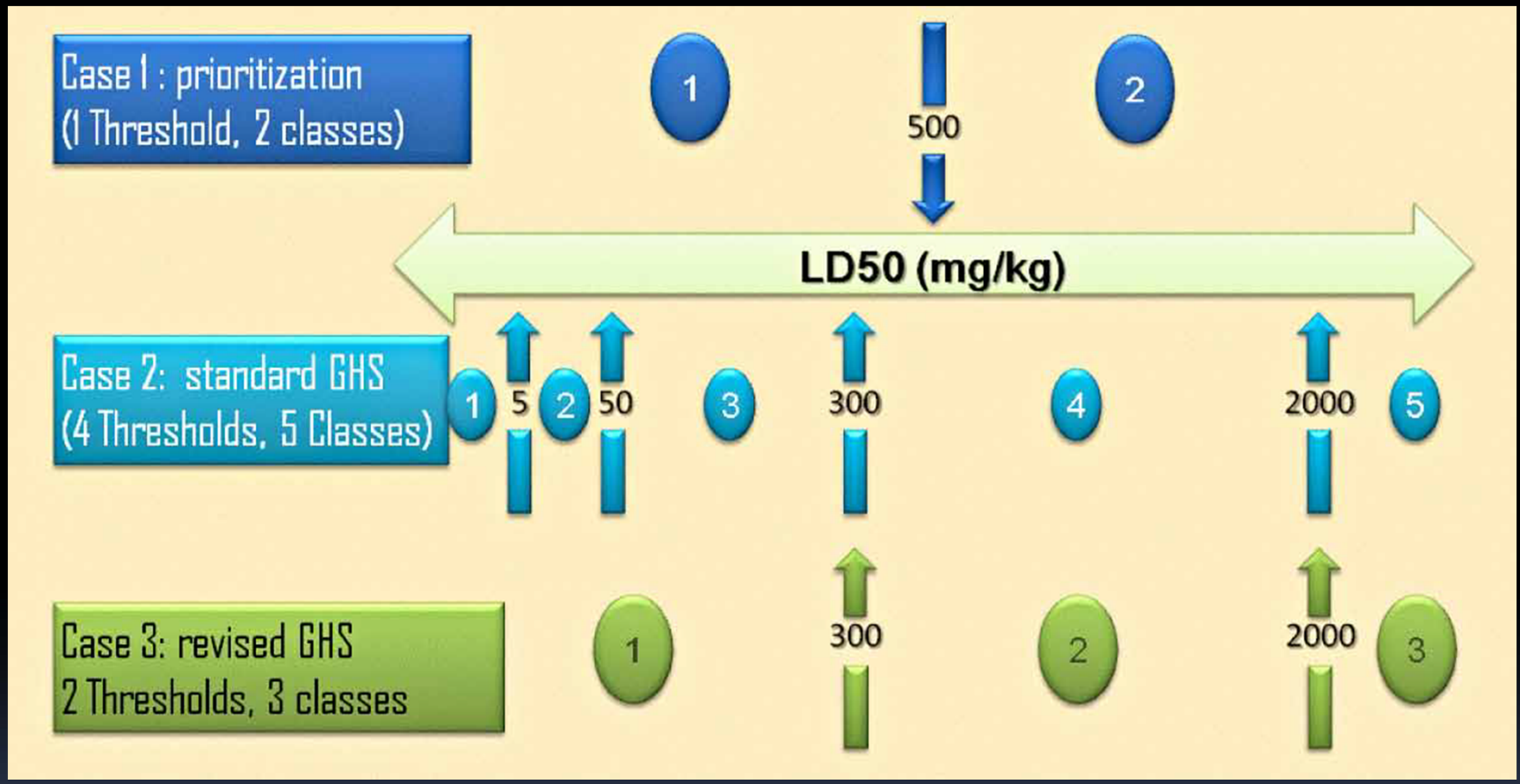
An In Vitro Acute LD50 Toxicity Model for Cosmetic Industry



Note, R et al. 2010 Presented at the World Congress on Alternatives to Animal Testing Montréal, August 2011

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Application of the Optimized Version of the In Vitro Acute LD50 Assay

- Early screening with specific LD50 thresholds: case 1
- Classification and labeling purposes (GHS categorization): case 2&3
- Change current classification systems for acute oral toxicity as suggested in the final document of the FP6 AcuteTox program: case 3

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Conclusions

- It is currently possible to predict in vivo toxicity (animals) with a cell-based model
- Approach includes functional endpoints for cell health, physical and chemical and pharmacokinetic properties
- Models are based initially on plasma concentrations that result in toxicity
- High level of concordance with in vivo toxicity
- An acute LD50 can be determined

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Future Improvements to the Model

- Develop a drug/chemical database with animal 28 or 90 day data, *in vitro* toxicity data, chemical properties
- Determine a calculation for NOAEL in humans
- Broaden the chemical space

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CeeTox Overview of Services

… a wide range of *in vitro* toxicology and safety assessment services

- **Systemic Toxicity Services**
  - CTOX Panel®
  - Acute Toxicity Screen
- ***In vitro* LD50 (acute tox)**
- **Dermal Toxicity**
  - Percutaneous Absorption
  - Corrosion
  - Irritation
  - Sensitization
  - Photo Toxicity
- **Ocular Toxicity**
- **Endocrine Disruption**
  - Estrogen receptor (ER) binding
  - Estrogen transcriptional activation
  - Androgen receptor (AR) binding
  - Steroidogenesis
  - Aromatase
- **Organ Specific Toxicity Models**
  - Heart (CardioTox®)
  - Kidney
  - Liver
- **CYP Induction/Inhibition**
- **Drug / Drug Interaction**
- **Target / Pathway Assays**
  - Acute Inflammatory Response
  - Apoptosis
  - Cell Proliferation
  - Cell Viability
  - Glutathione Homeostasis
  - Hepatobiliary Toxicity
  - Kidney Collecting Duct Toxicity
  - Kidney Distal Tubular Toxicity
  - Kidney Proximal Tubule Toxicity
  - Lipidosis
  - Membrane Integrity
  - Metabolism
  - Mitochondrial Function

Acknowledgments

CeeTox staff

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