Incorporating modernized approaches and data sources to assess temporal exposures: Considerations across the source to outcome continuum

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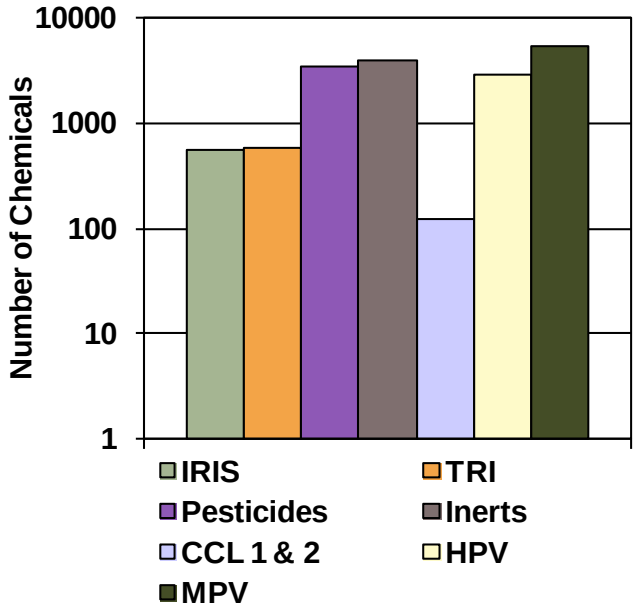
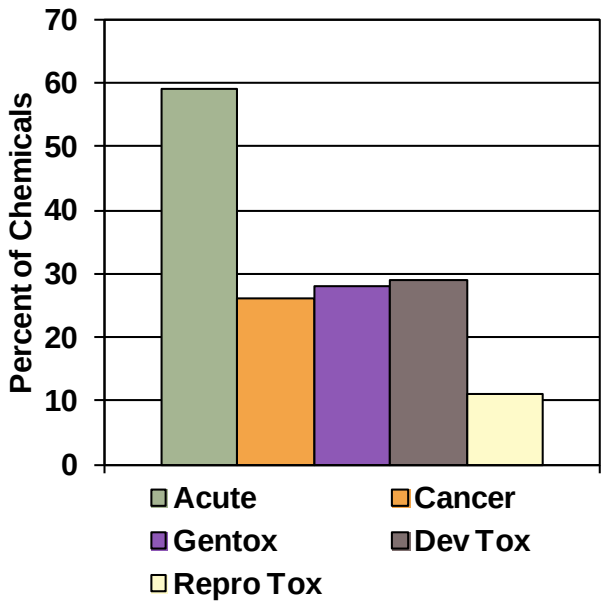
ScitoVation

January 29, 2016

ScitoVation
INNOVATIVE CELL BASED SCIENCE

Considering Alternative Testing Strategies to Modernize Toxicity Testing





Traditional testing paradigm does not incorporate advances in technology or biological understanding

... and cannot efficiently assess safety of all the existing chemicals or keep pace with those being developed

Judson, et al *EHP* (2010)

Global Push for Modernization of Testing

Multiple Drivers; Similar Path Forward

114TH CONGRESS
1ST SESSION

H. R. 2576

To modernize the Toxic Substances Control Act, and for other purposes.

IN THE HOUSE OF REPRESENTATIVES

MAY 26, 2015

Mr. SHIMKUS (for himself, Mr. UPTON, Mr. PALLONE, and Mr. TONKO) introduced the following bill; which was referred to the Committee on Energy and Commerce

A BILL

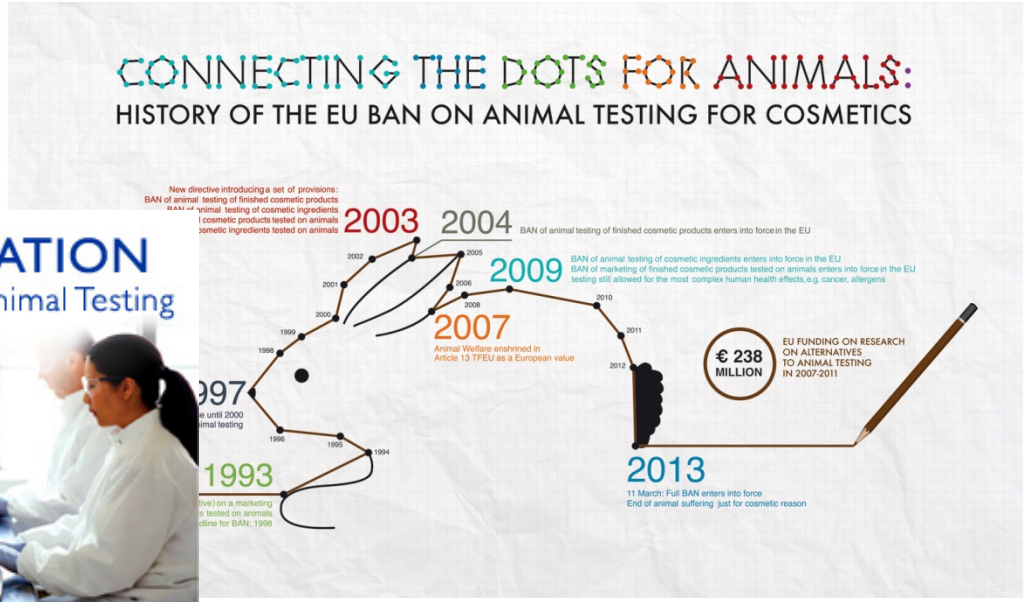
To modernize the Toxic Substances Control Act, and for

Calendar No. 121

114TH CONGRESS
1ST SESSION

S. 697

To amend the Toxic Substances Control Act to reauthorize and modernize that Act, and for other purposes.



Talk Outline

- Leveraging In Vitro Tools in Toxicity Testing
- Incorporating Dosimetry and Exposure with In Vitro Data to provide a Risk-Based Context
- Integrating Modeling and In Vitro Tools to Assess Interindividual Variability and Life-Stage Differences

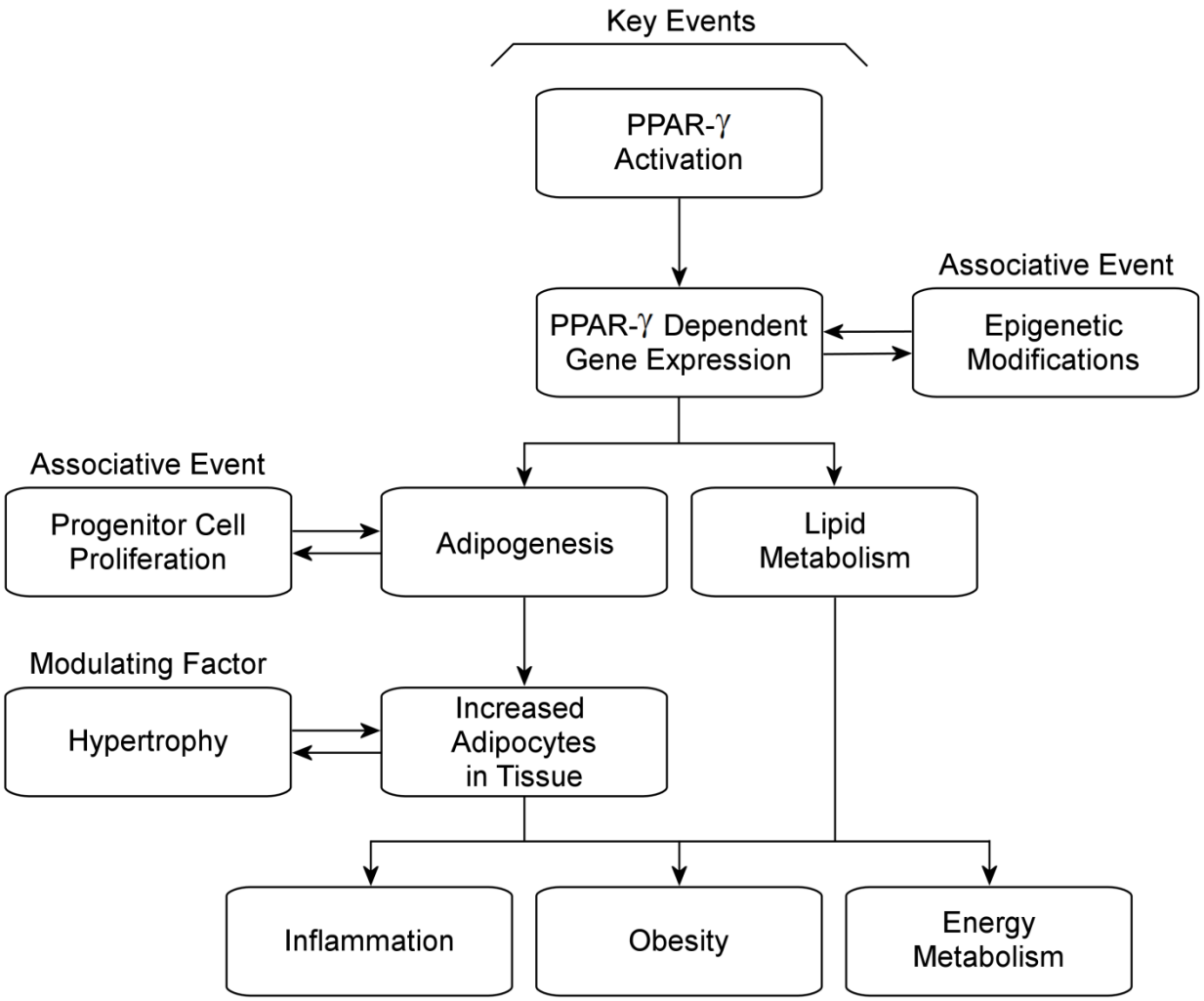
"Fit-for-Purpose" *in vitro* Assays for Toxicity Testing

Toxicological endpoint	Assay
Metabolic Disease	Human Adipogenesis
	Human Liver Steatosis
Liver Carcinogenesis	Human/Rodent Hepatocyte Proliferation
	Human/Rodent Nuclear Receptor Translocation
Developmental Toxicity	Human iPSC Differentiation
Endocrine Disruption	Rodent Thyroid Metabolism
	Human Uterine Cell Proliferation/ER Activation
	Rodent Steroidogenesis
Genotoxicity	Human DNA Damage Foci Formation
Oxidative Stress	Human NRF2 Activation
	Human roGFP ROS Reporter

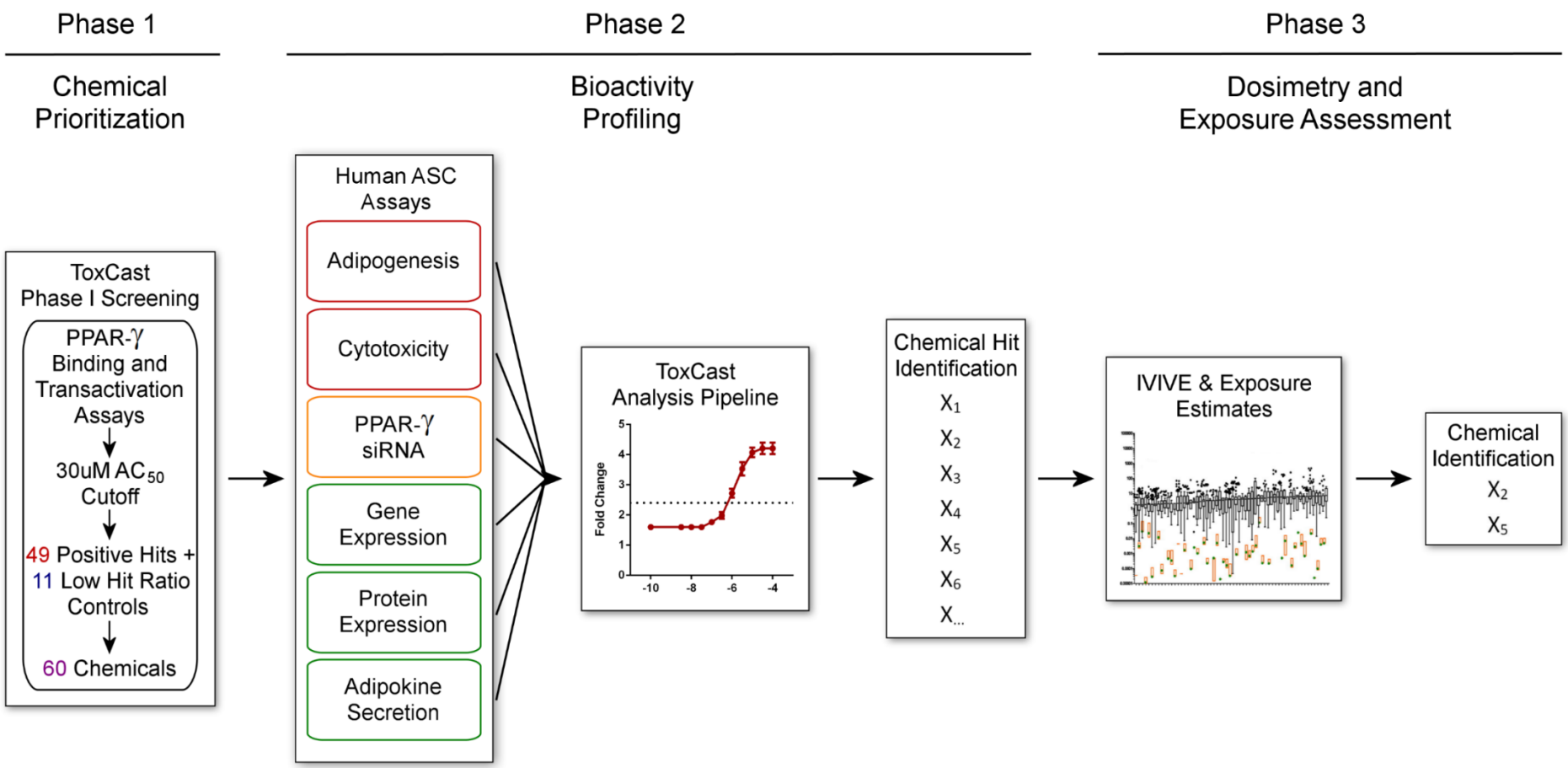
Goal: Establish *in vitro* models that are predictive of human health outcomes

- Use primary and stem cell models, preferably of human origin
- Apply new technologies to acquire quantitative concentration-response data
- Enable medium-to-high throughput screening
- Design orthogonal assay sets for data-driven decision making
- Incorporate dosimetry and exposure relevance
- Apply data analysis tools from NTP partner agencies

PPARG Dependent Adipogenesis Mode-of-Action Framework

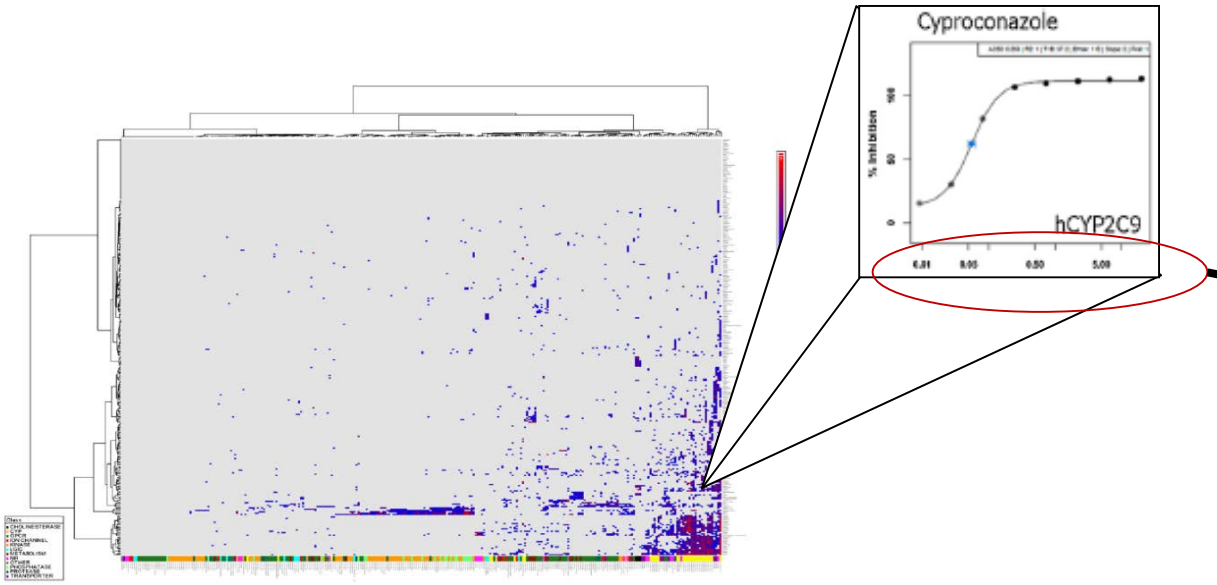


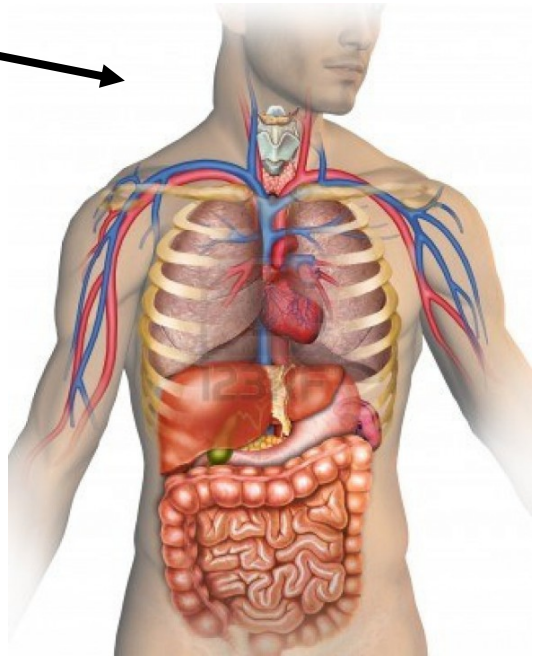
Integrated Approach to Screening for *PPARG* Dependent Adipogenesis



Challenges of *In Vitro* Toxicity Testing Data

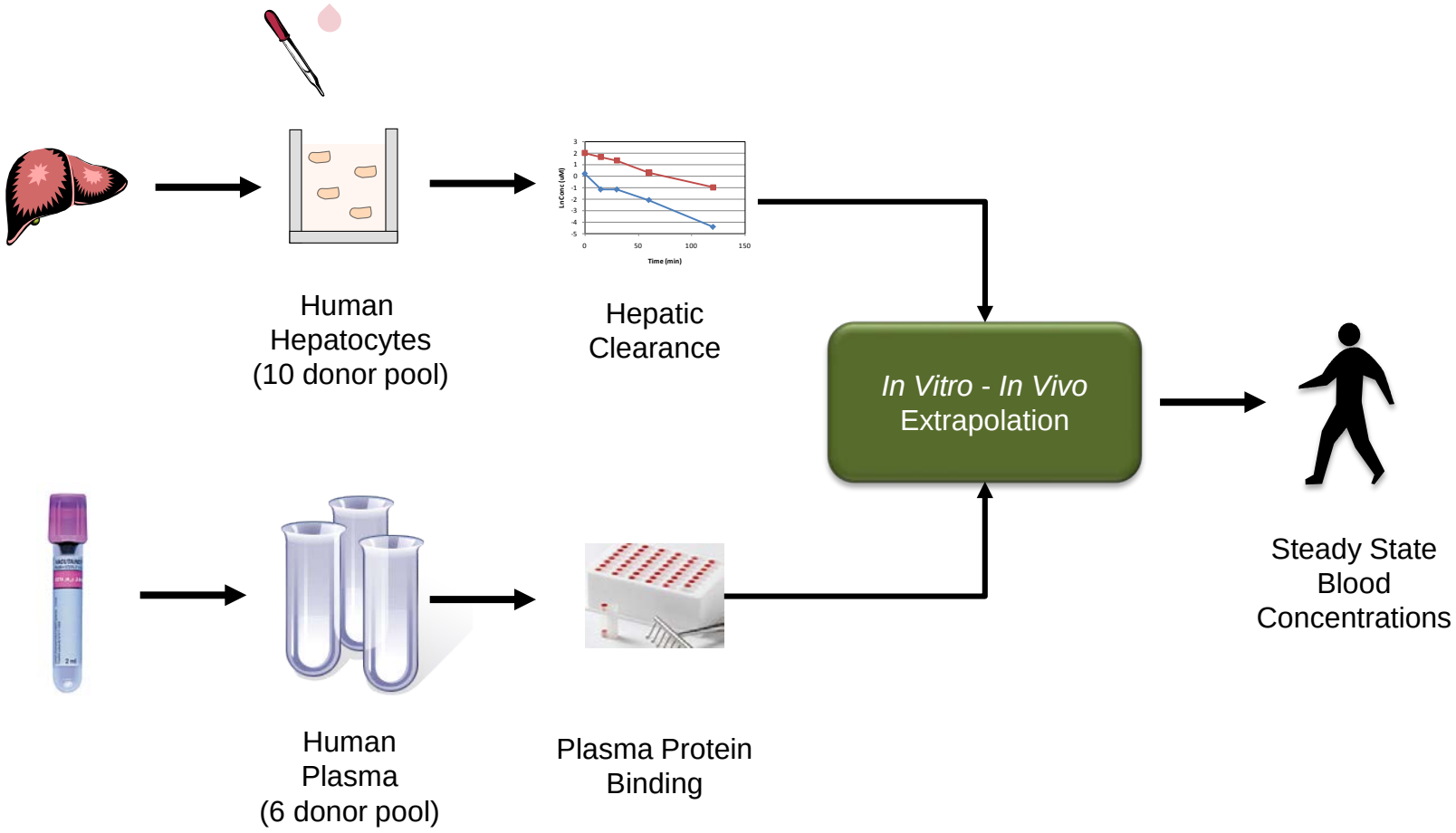
Difficulty Translating Nominal Testing Concentrations into *In Vivo* Doses





Knudsen *et al.* Toxicology 282:1-15, 2011

— In Vitro-In Vivo Extrapolation —
Modeling *In Vivo* Pharmacokinetics Using *In Vitro* Assays



— In Vitro-In Vivo Extrapolation —
Modeling *In Vivo* Pharmacokinetics Using *In Vitro* Assays

In Vitro - In Vivo Extrapolation →

$$[Conc]_{SS} = \frac{\text{Dose Rate} * \text{Body Weight}}{CL_{WholeBody}}$$

$$CL_{WholeBody} = CL_R + CL_H$$

- 100% Oral bioavailability assumed for both CL_R and CL_H
- **Kinetics are assumed to be linear**

- CL_R: renal clearance (L/hr)
- $CL_{H:}$ hepatic clearance (L/hr)
- $Cl_{int:}$ intrinsic clearance (L/hr)
- GFR: glomerular filtration rate (L/hr)
- F_{uB}: fraction unbound in blood
- Q_L: hepatic blood flow (L/hr)
- HPGL: hepatocytes per gram liver
- $V_{L:}$ volume of liver (g)

$$CL_R = F_{UB} * GFR$$ *where GFR ≈ 6.7 L/hr*

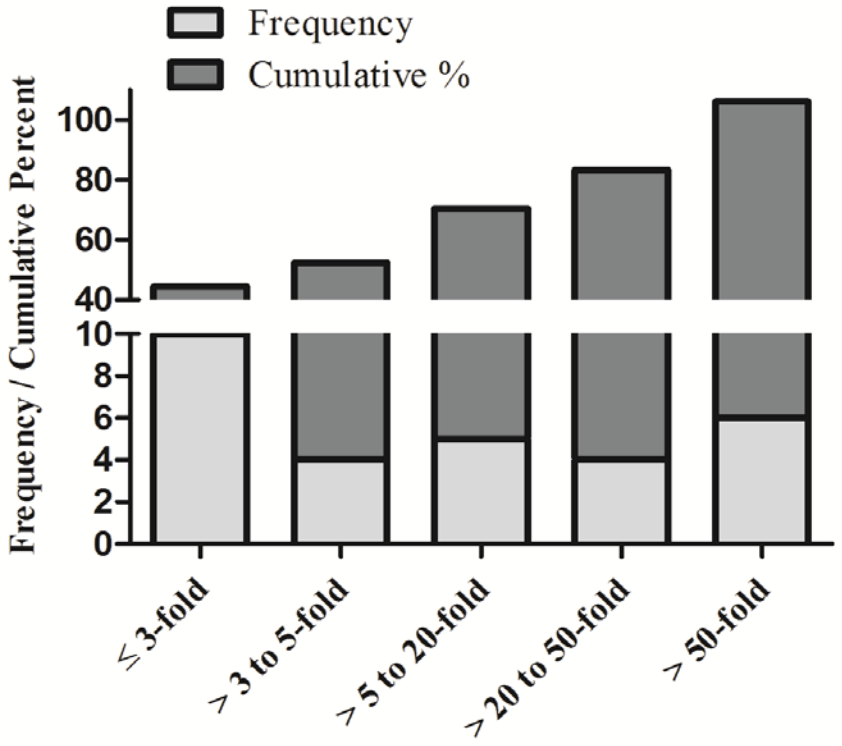
$$CL_H = \frac{F_{UB} * Q_L * CL_{Int}}{Q_L + F_{UB} * CL_{Int}}$$ *where Q_L ≈ 90 L/hr*

$$CL_{Int} = HPGL * V_L * CL_{invitro}$$ *where HPGL ≈ 137 million cells/g*

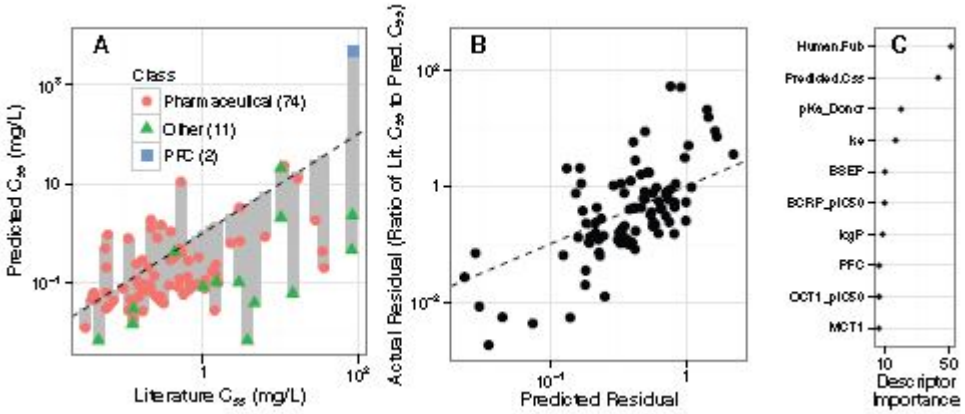
where V_L ≈ 1820 g

How good are we at predicting *in vivo* C_{ss}?

27 Chemicals:
~60% are within 10-fold of *in vivo* C_{ss} values
~80% are within 20-fold of *in vivo* C_{ss} values



Toxicokinetic Triage for Environmental Chemicals



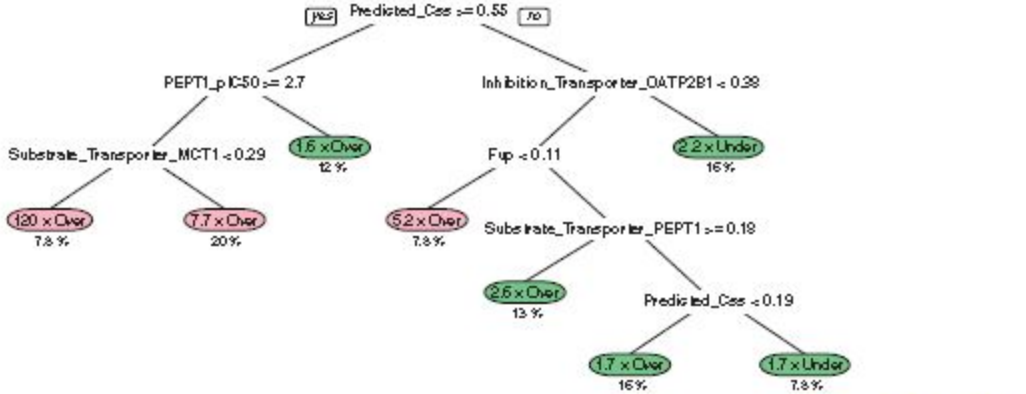
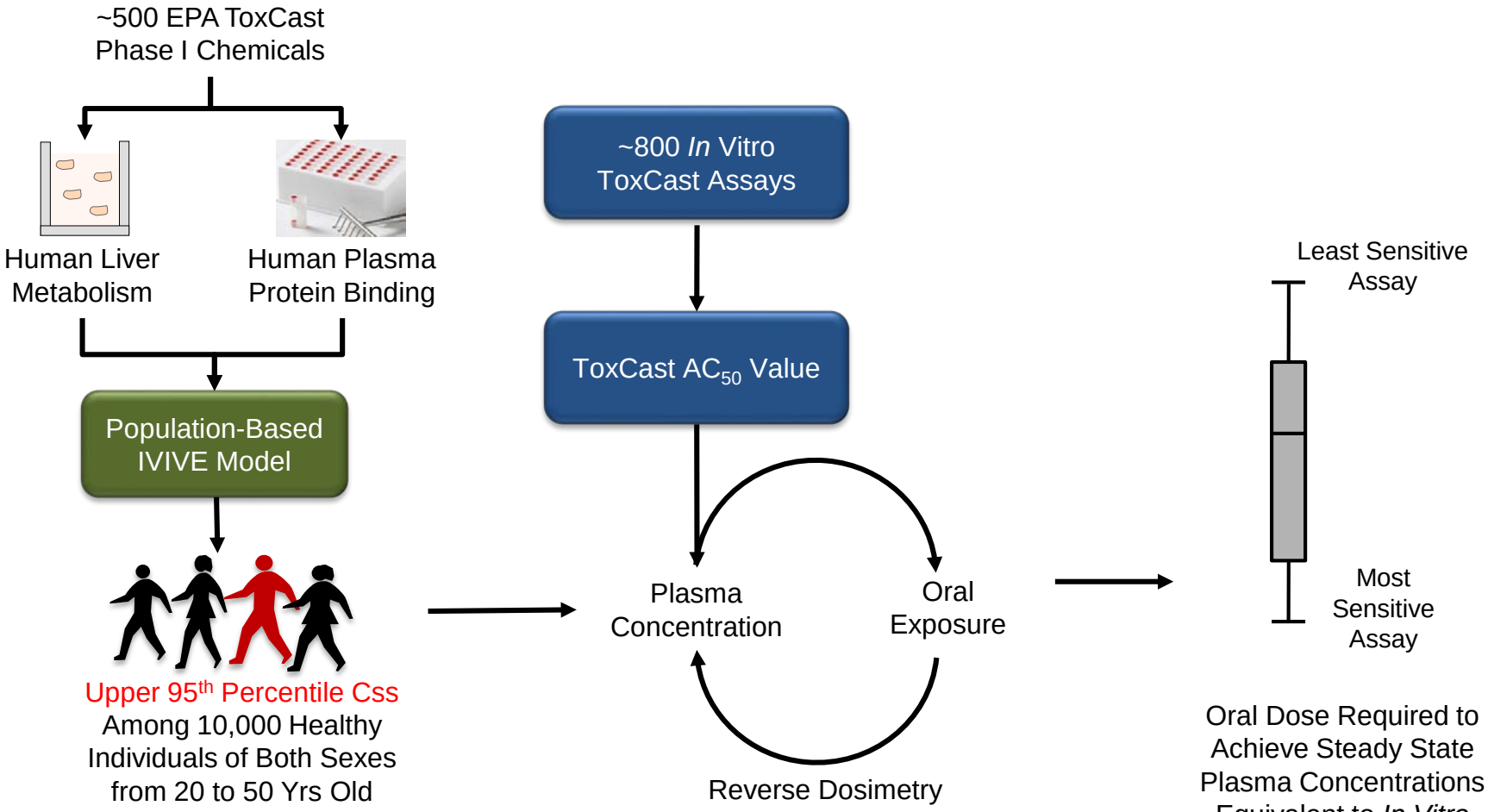


FIG. 5. A recursive partitioning regression tree was used to classify the discrepancy between the C_{ss} predicted from in vitro data and the in vivo C_{ss} (Obach et al., 2008; Wetmore et al., 2012). Each "leaf" of the tree shows a group of chemicals for which HTTK either overestimates C_{ss} (making conservative predictions) or underestimates C_{ss}. For all but 3 groups, the predictions are on the order of the observed C_{ss} (approximately within a factor of 3.2× greater or lesser). For the other 3 groups, the C_{ss} is 5.2×, 7.7×, and 120× overestimated. The dashed line indicates the identity (perfect predictor) line.

Wambaugh *et al.*, *Tox Sci.*, 2015

Integrating Human Dosimetry and Exposure with the ToxCast *In Vitro* Assays



Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.*, 2012
Wetmore *et al., Tox Sci.*, 2015

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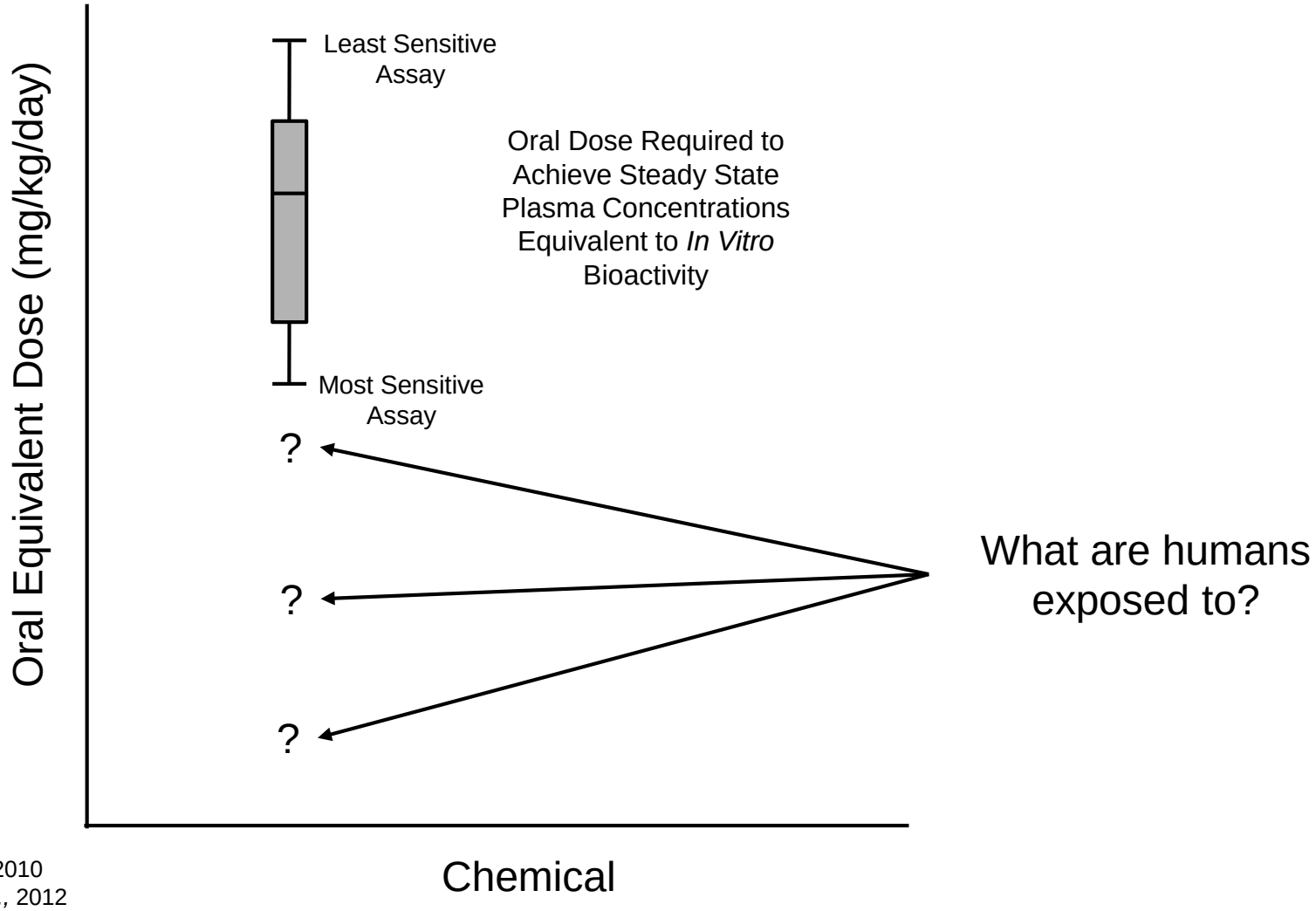
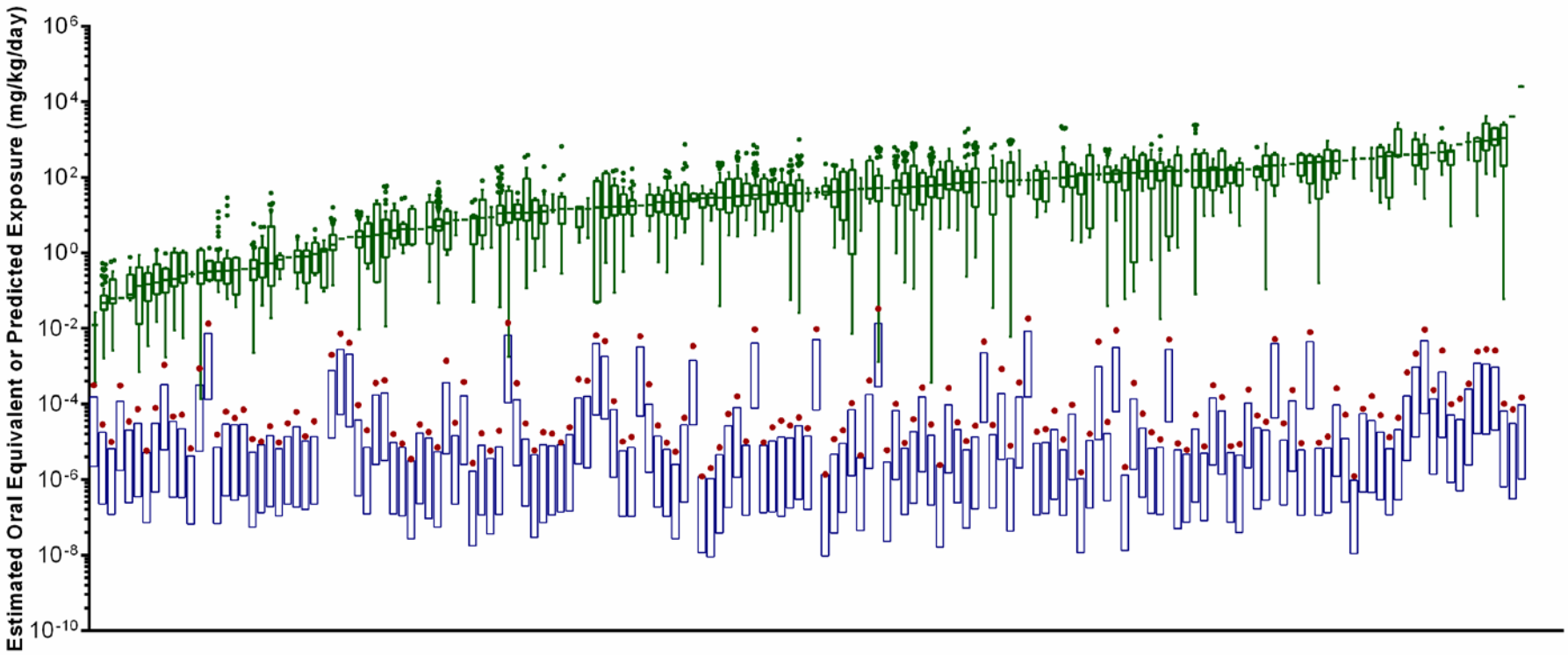


Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012
Wetmore *et al., Tox Sci.,* 2015

Incorporating Dosimetry-Adjusted ToxCast Bioactivity Data with HT ExpoCast Predictions



Wetmore *et al.*, Tox. Sci, 2015

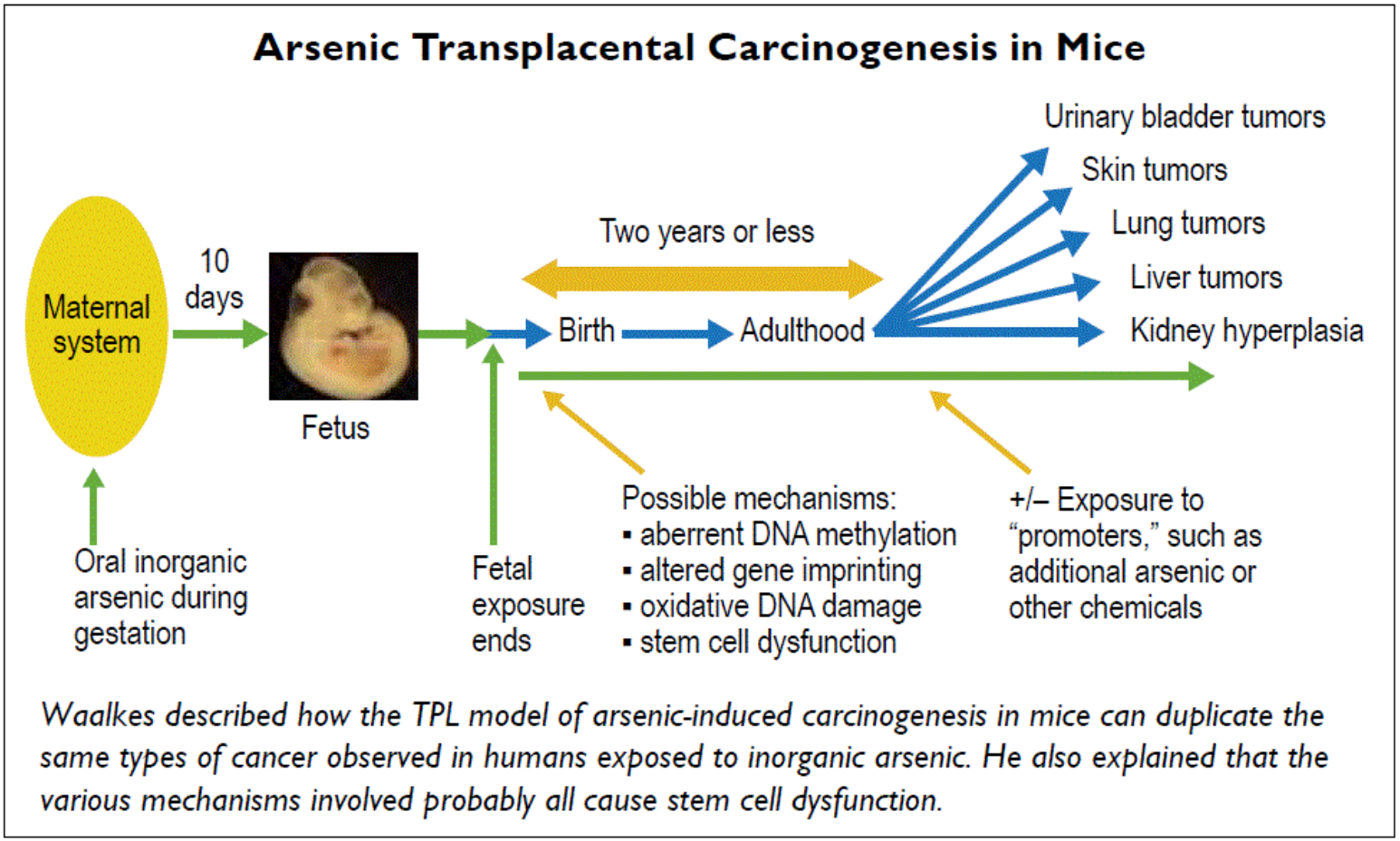
Capturing Exposures Across a Life-Course

Inter-individual variability

Developmental differences across life-stages

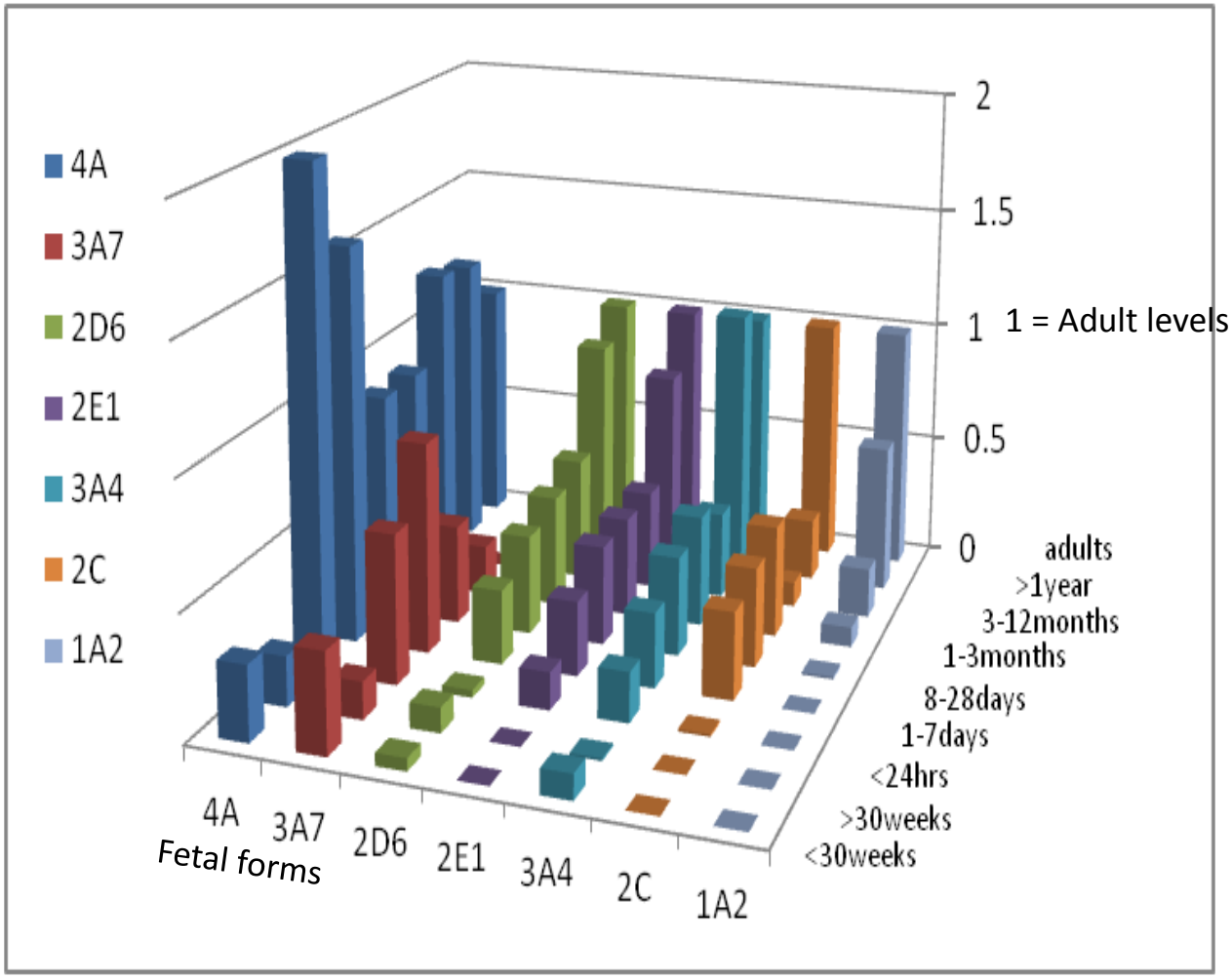
Genetic differences across ethnicities

Physiologic differences across across life-stages and groups



Waalkes described how the TPL model of arsenic-induced carcinogenesis in mice can duplicate the same types of cancer observed in humans exposed to inorganic arsenic. He also explained that the various mechanisms involved probably all cause stem cell dysfunction.

Ontogenies of XMEs in Children



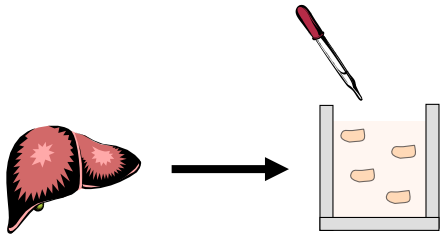
- CYPs rapidly developing in first 6 months of life;
- Best studied of all XMEs (more data needed on others)
- Challenges: variability extremely high – impossible to discern interindividual variability from variability due to rapidly developing system

Adapted from Cresteil et al., 1998

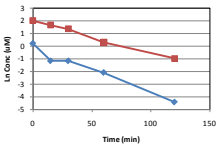
TK Variability in Children

Developmental Feature	Relevant Life-Stage	Impact on TK
Body composition: lower lipid, greater water content	Birth through 3 months	↓ partitioning and retention of lipid-soluble cmpds ↑ V_d for water soluble cmpds
Larger liver:body weight ratio	Birth through 6 yr (largest ratios, birth-2yr)	↑ Hepatic extraction/metabolite clearance ↑ potential metabolic activation
Immature Phase I/II enzyme fucntionality	Birth through 1 yr (largest differences in first 2 months)	↓ metabolic clearance, activation ↓ removal of activated metabolites
Larger brain:body weight ratio; greater CNS blood flow; higher BBB permeability	Birth through 6 yr (largest differences in first 2 yr)	↑ CNS exposure, particularly for water soluble agents normally impeded by BBB
Immature renal function	Birth through 2 months	↓ elimination of renally cleared chemicals/metabolites
Limited serum protein binding capacity	Birth through 3 months	↑ potential, free toxicant ↑ distribution of chemicals normally bound/unavailable

Population-based *In Vitro-In Vivo* Extrapolation

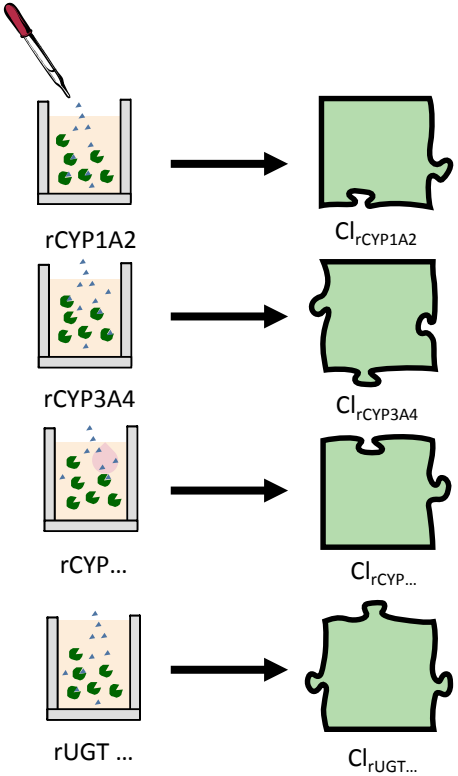


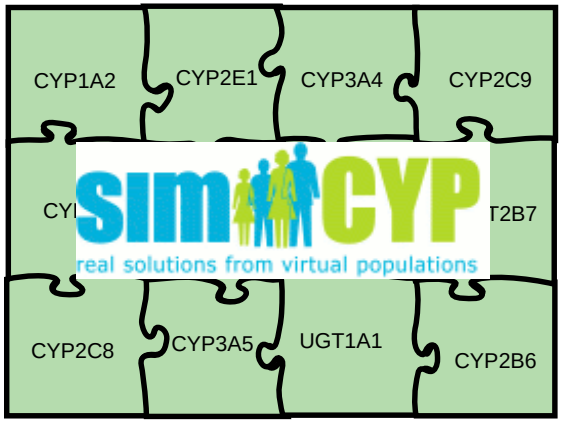
Primary Hepatocytes



Hepatic Clearance
$Cl_{in\ vitro}$

Plasma C_{ss}
General Population





Intrinsic Clearance Rates

Plasma C_{ss} for:

Neonates

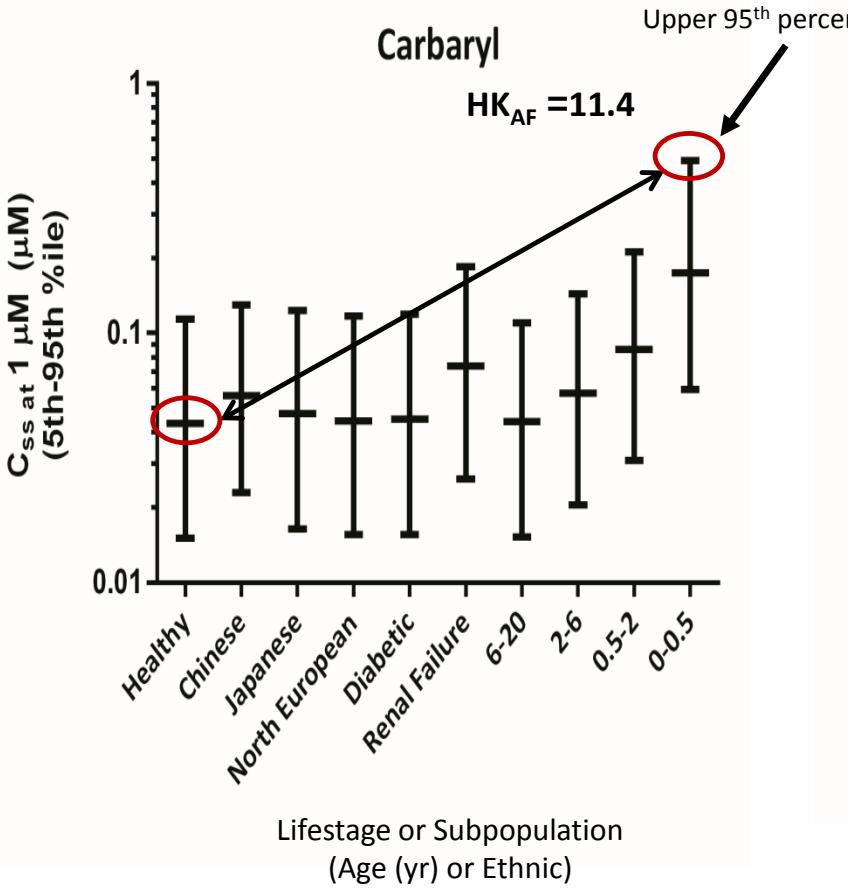
Asians

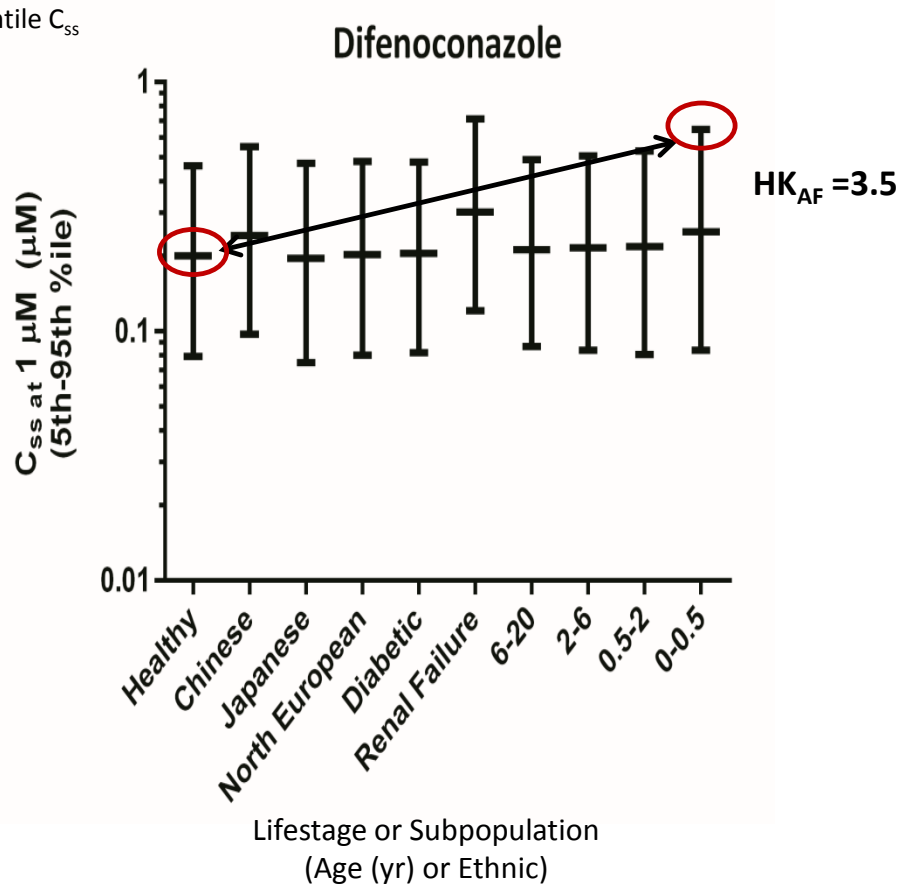
Northern Europeans

Children

And so on…

Comparison of C_{ss} Values Derived Across Multiple Lifestages and Subpopulations



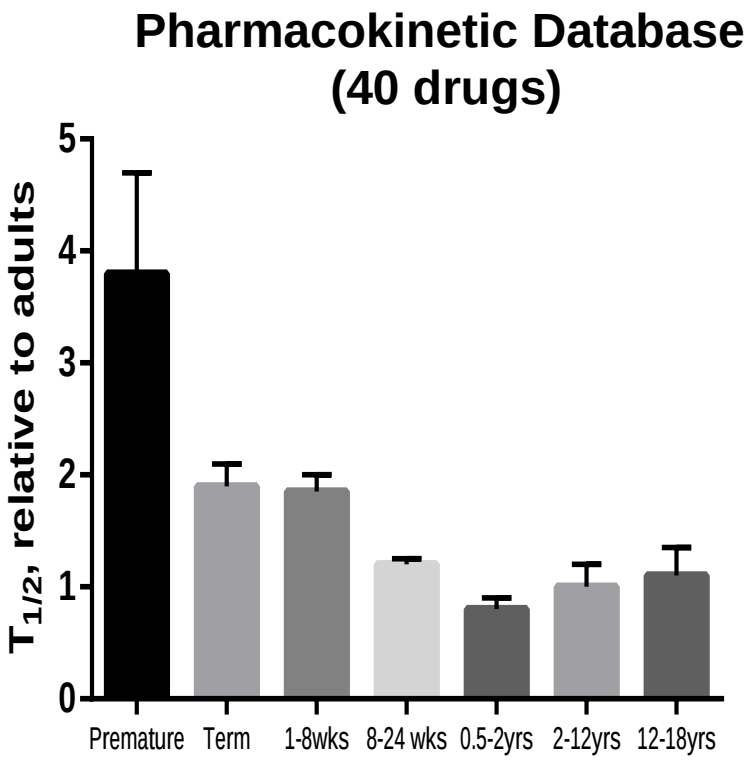


HK_{AF}: human toxicokinetic adjustment factor

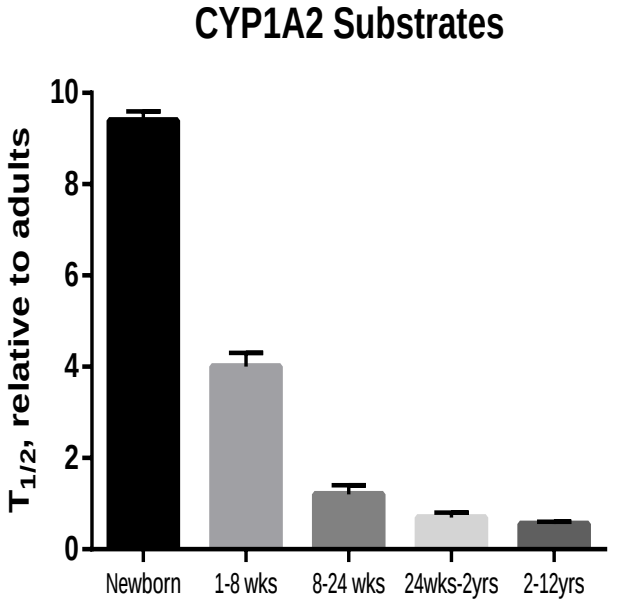
Estimated Chemical-Specific Toxicokinetic Adjustment Factors

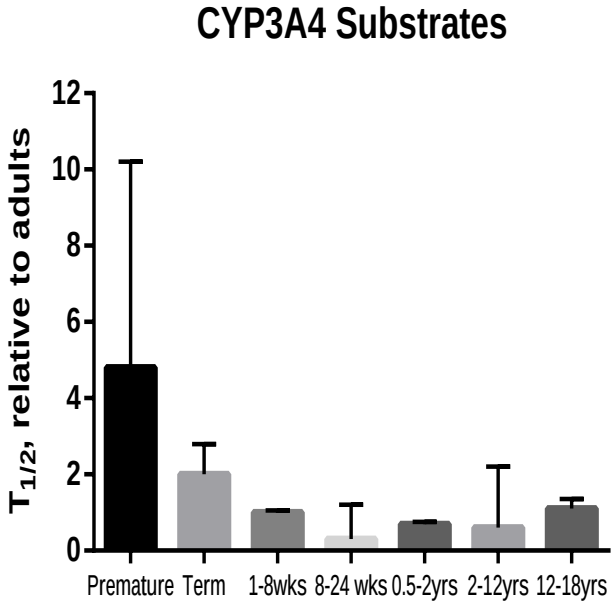
Chemical	Median C_{ss} for Healthy Population	95th Percentile C_{ss} for Most Sensitive	Most Sensitive	Estimated HK_{AF}	% Contribution of Isozyme Differences to Average HK_{AF}
Acetochlor	0.026	0.15	Neonatal	6.7	86
Azoxystrobin	0.099	0.66	Neonatal	6.7	86
Bensulide	0.241	0.97	Neonatal	4.0	79
Carbaryl	0.043	0.49	Neonatal	11.4	87
Difenoconazole	0.201	0.49	Renal Insufficiency	3.5	99
Fludioxonil	0.38	4.37	Neonatal	11.5	87
Haloperidol	0.029	0.14	Neonatal	4.9	83
Lovastatin	0.001	0.009	Neonatal	6.5	90
Tebupirimfos	0.107	0.38	Renal Insufficiency	3.5	15

TK Variability in Children Clearance Rates across Drugs

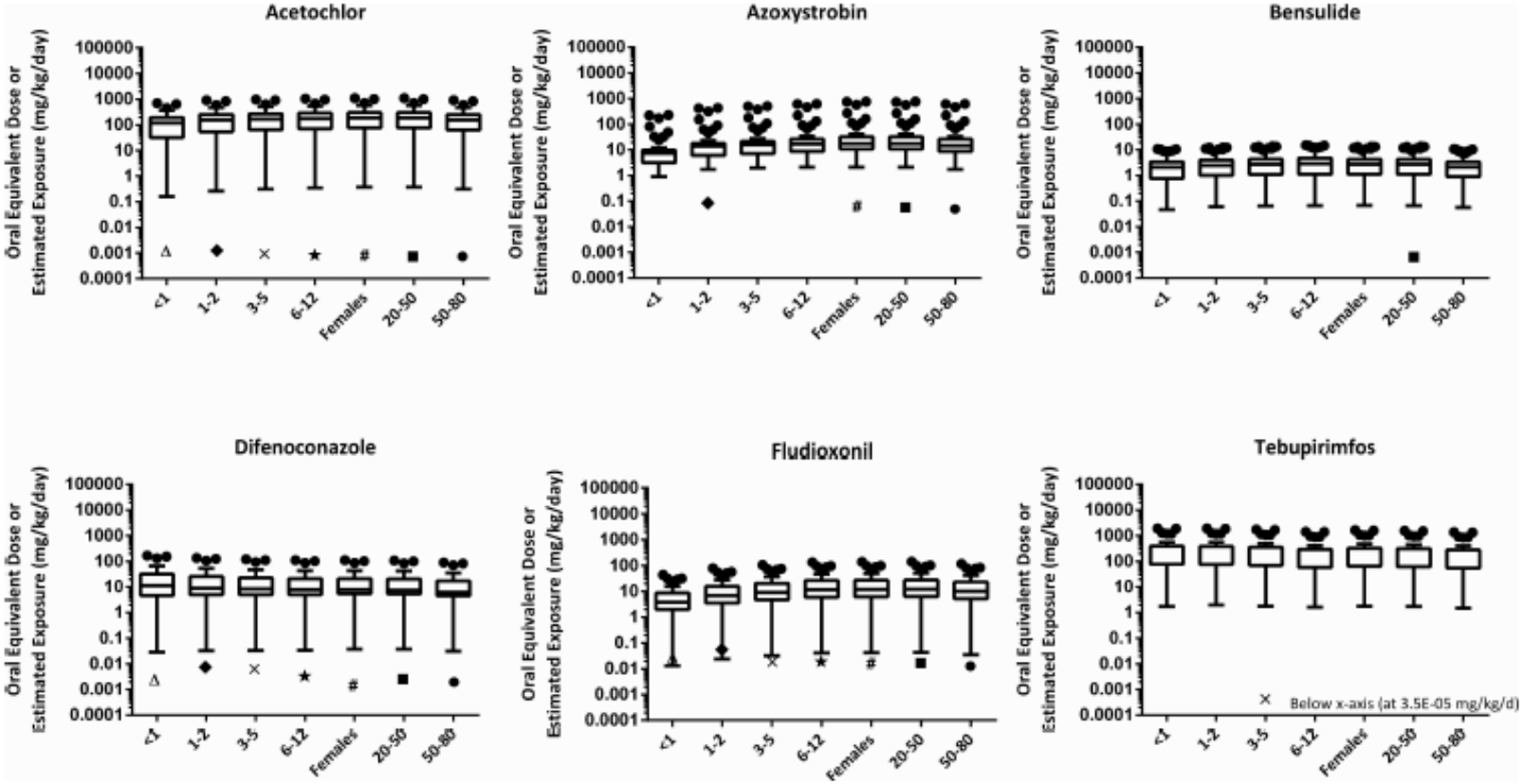


Adapted from Ginsberg et al., 2004

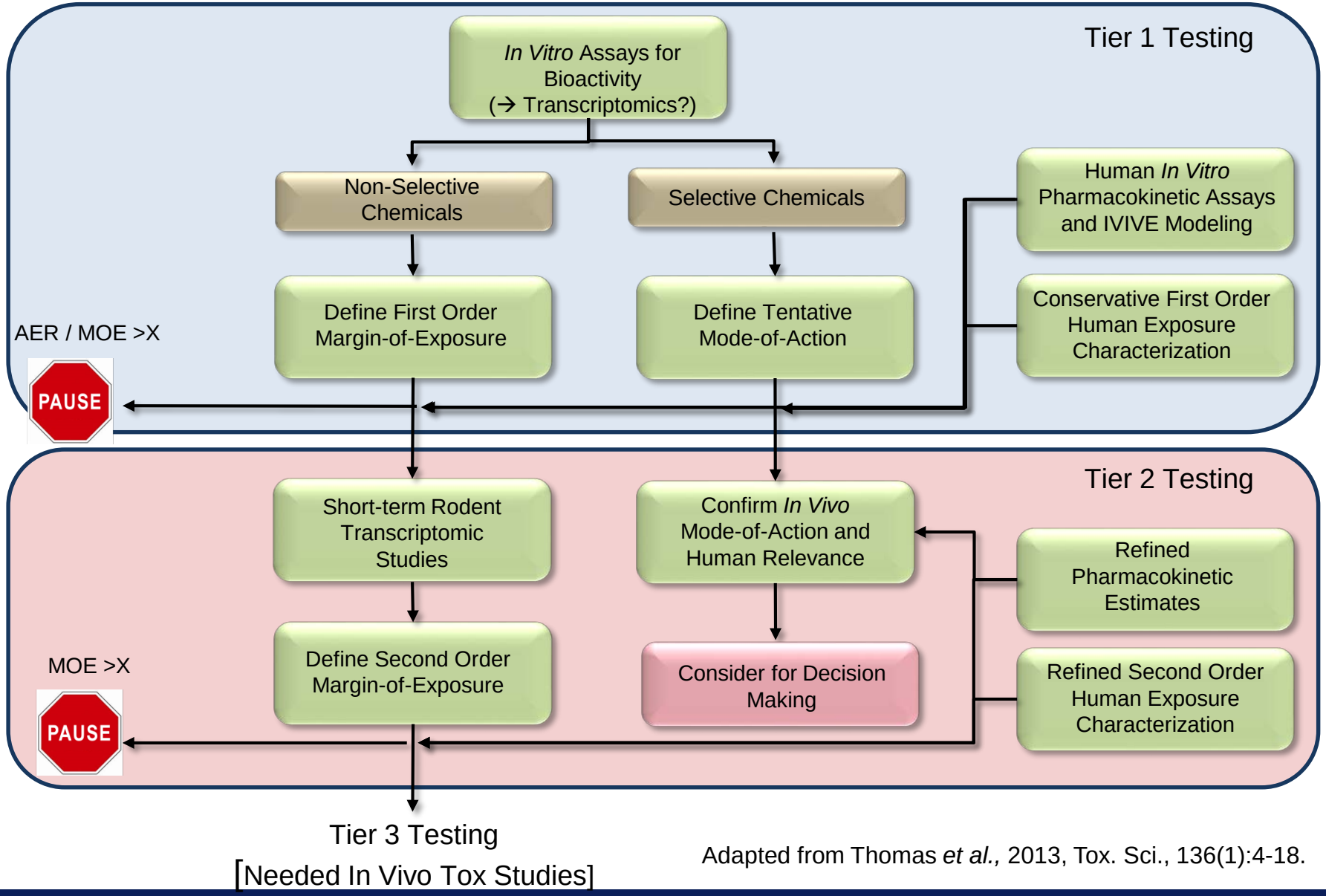




Matching Oral Equivalent Doses and Exposure Estimates for Subpopulations



Utility in a Tiered Decision-Making Framework



Adapted from Thomas *et al.,* 2013, Tox. Sci., 136(1):4-18.

Conclusions

- When key events known for apical outcomes, fit-for-purpose in vitro tools hold potential to guide in toxicological assessments.
- Incorporating in vitro assay data with IVIVE tools for dosimetric adjustment has enabled a shift from a hazard-based to a risk-based interpretation of in vitro data.
- IVIVE effort to evaluate PK variability in a manner that could 1) identify sensitive populations and 2) replace use of default safety factors in risk assessment.
- Current in vitro – in vivo assessments for environmental pollutants point to need for tools trained against relevant space for prediction refinement.
- Although many gaps and considerations exist in in vitro assay development and IVIVE, many of these can – and are – being addressed.

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