THE HAMNER INSTITUTES FOR HEALTH SCIENCES
WHERE GREAT MINDS & MEDICINE MEET

Incorporating Population Variability and Sensitive Subpopulations into Dosimetry for High-Throughput Toxicity Testing

Computational Toxicology Communities of Practice

October 31, 2013

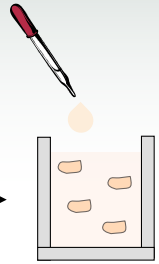
Barbara A. Wetmore

Institute for Chemical Safety Sciences

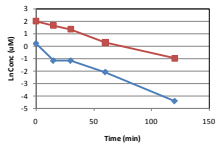
The Hamner Institutes for Health Sciences

Setting the Stage…

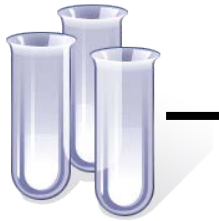
Incorporating Dosimetry with High-Throughput Screening Data



Human Hepatocytes (10 donor pool)



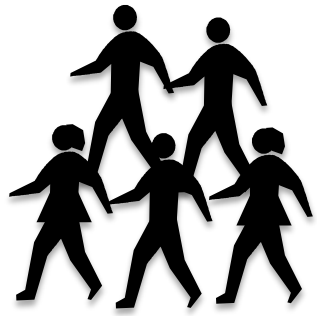
Hepatic Clearance



Human Plasma (6 donor pool)

Plasma Protein Binding

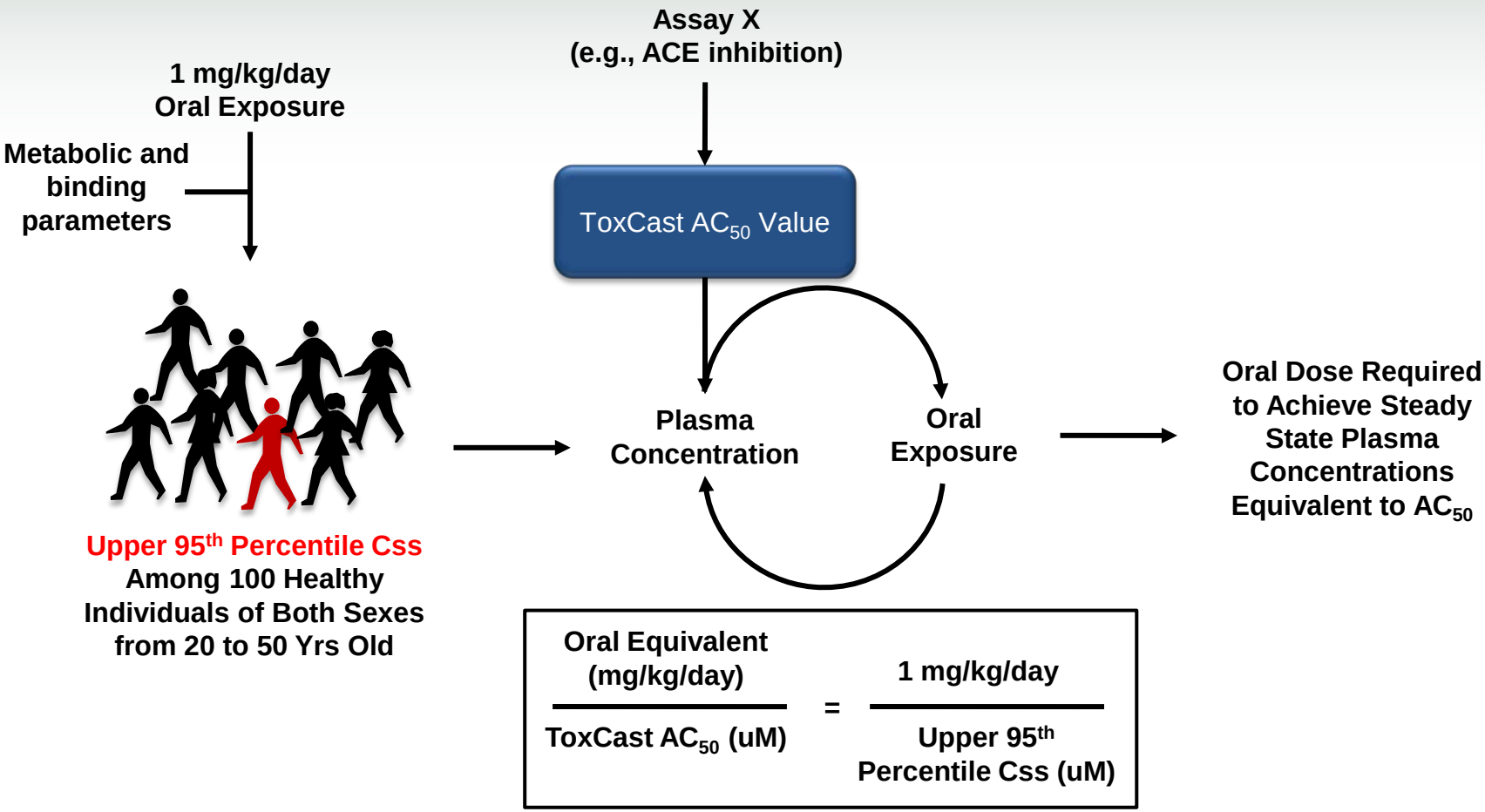
Population-Based *In Vitro*-to-*In Vivo* Extrapolation



Plasma Concentration at Steady State for 100 Healthy Individuals of Both Sexes from 20 to 50 Yrs Old

Setting the Stage…

Using Reverse Dosimetry to Estimate Population-Based Oral Equivalent Doses



Incorporating Dosimetry and Exposure with HTS Data to Better Inform HT Risk Assessment

Incorporating Human Dosimetry and Exposure into High-Throughput *In Vitro* Toxicity Screening

Daniel M. Rotroff,*,† Barbara A. Wetmore,‡ David J. Dix,* Stephen S. Ferguson,§ Harvey J. Clewell,‡ Keith A. Houck,* Edward L. LeCluyse,§ Melvin E. Andersen,‡ Richard S. Judson,* Cornelia M. Smith,§ Mark A. Sochaski,‡ Robert J. Kavlock,* Frank Boellmann,‡ Matthew T. Martin,* David M. Reif,* John F. Wambaugh,* and Russell S. Thomas‡,[1]

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Integration of Dosimetry, Exposure, and High-Throughput Screening Data in Chemical Toxicity Assessment

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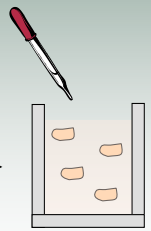
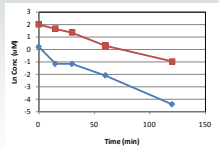
Dosimetry and Exposure Strategy Limited to General Population



Primary Hepatocytes



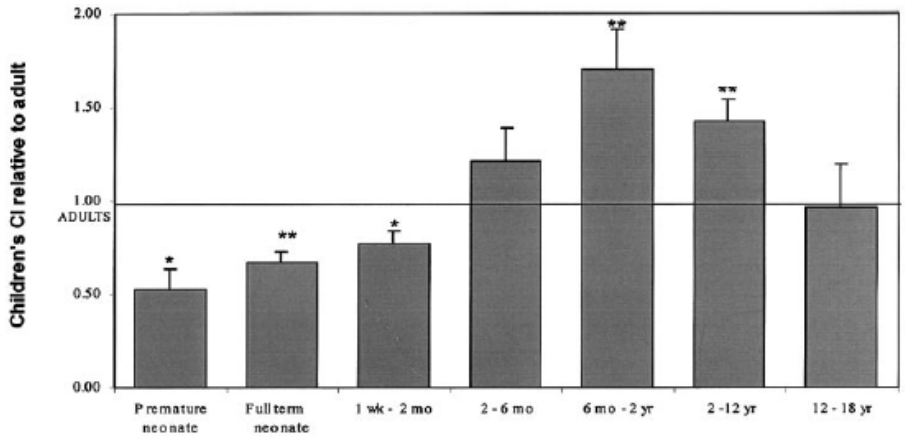
Hepatic Clearance
$Cl_{in\ vitro}$

Plasma C_{ss}
General Population

Plasma C_{ss}
General Population

≠

The Impact of Population Variability on Risk Assessment

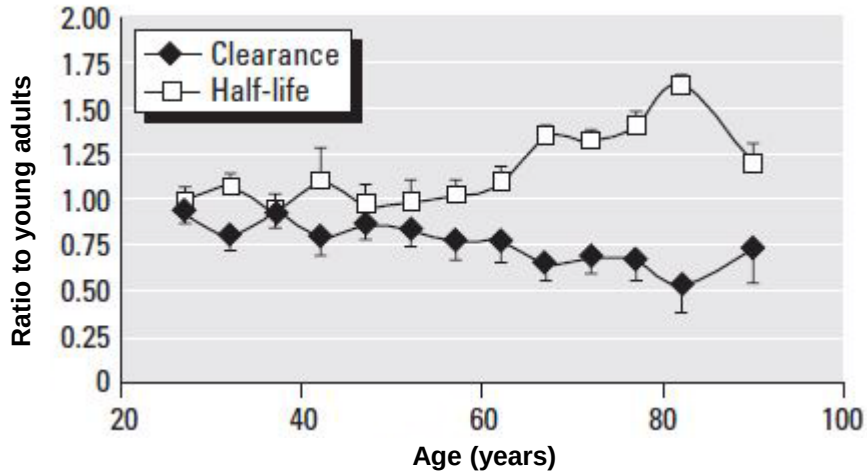


Clearance results for full database (27 substrates).

from Ginsberg *et al*., 2002, *Toxicol. Sci.*, 66, 185-200.

Clearance differences span across multiple juvenile subpopulations…

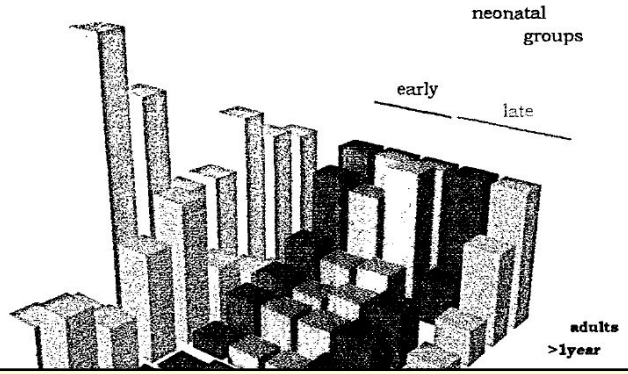
… and geriatric subpopulations.



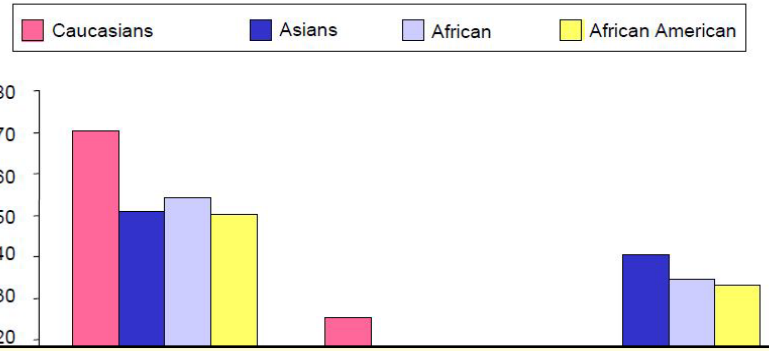
from Ginsberg *et al*., 2005, *Environ. Health Persp.*, 113, 1243-49.

The Impact of Population Variability on Risk Assessment

Ontogeny of Human Hepatic CYP Isozymes

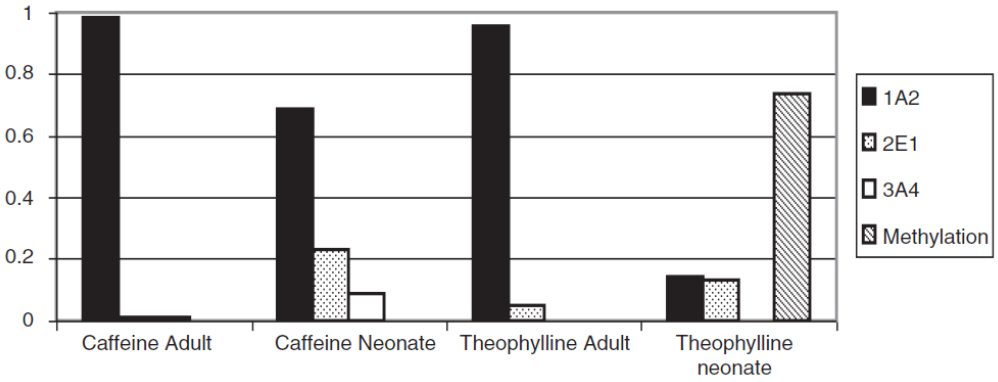


Frequency for CYP2D6 alleles classified as functional, non-functional and reduced functioning for various subpopulations.



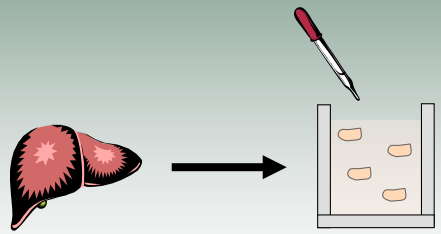
Sole reliance on pharmacokinetic data for a "generic" population could lead to a significant underestimation of risk to a susceptible subpopulation

Percent contribution of individual CYPs to total hepatic clearance of xanthines.



Ginsberg *et al.*, 2004, *J. Toxicol. Env. Health Pt. A,* 67:297-329.

Population-based *In Vitro*-to-*In Vivo* Extrapolation



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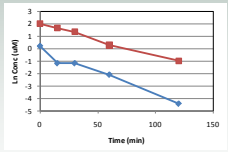
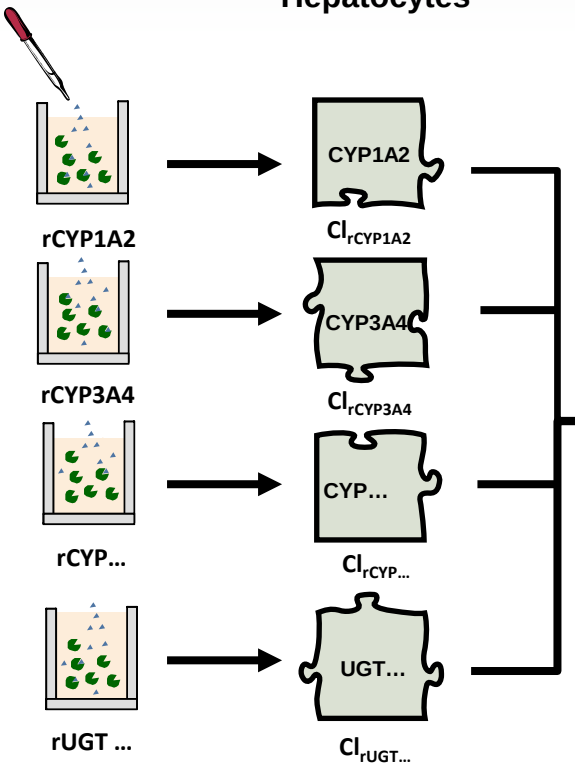
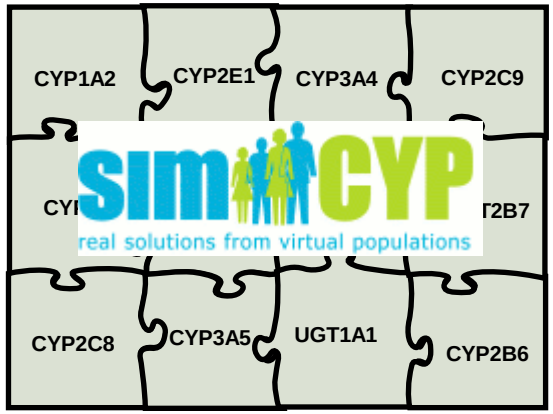


Hepatic Clearance
$Cl_{in\ vitro}$

Plasma C_{ss}
General Population





Intrinsic Clearance Rates

Plasma C_{ss} for:

Neonates

Asians

Northern Europeans

Children

And so on…

Incorporating Recombinant Phase I and II Enzyme Data into IVIVE Modeling

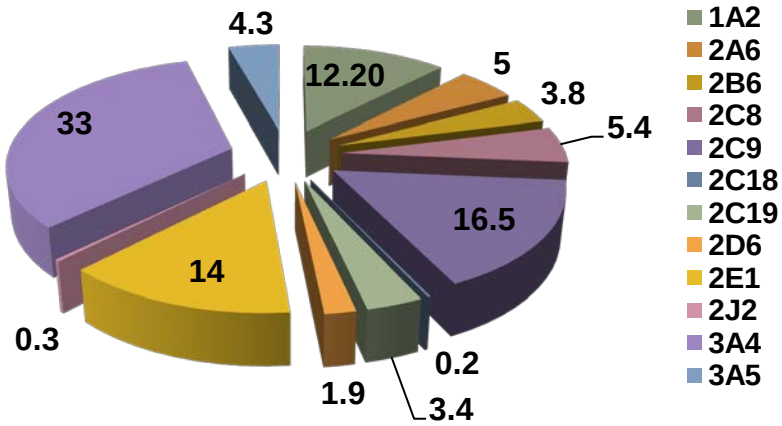
Scaling rCYP Data to HLM using intersystem extrapolation factors

$$ISEF = \frac{Cl_{int,\,HLM}\ (uL\ /\ min\ /\ mg\ protn)}{Cl_{int,\,rCYP}\ (uL\ /\ min{*}pmol\ P450)\ \times\ HLM\ CYP\ abundance\ (pmol\ P450\ /\ mg\ protn)}$$

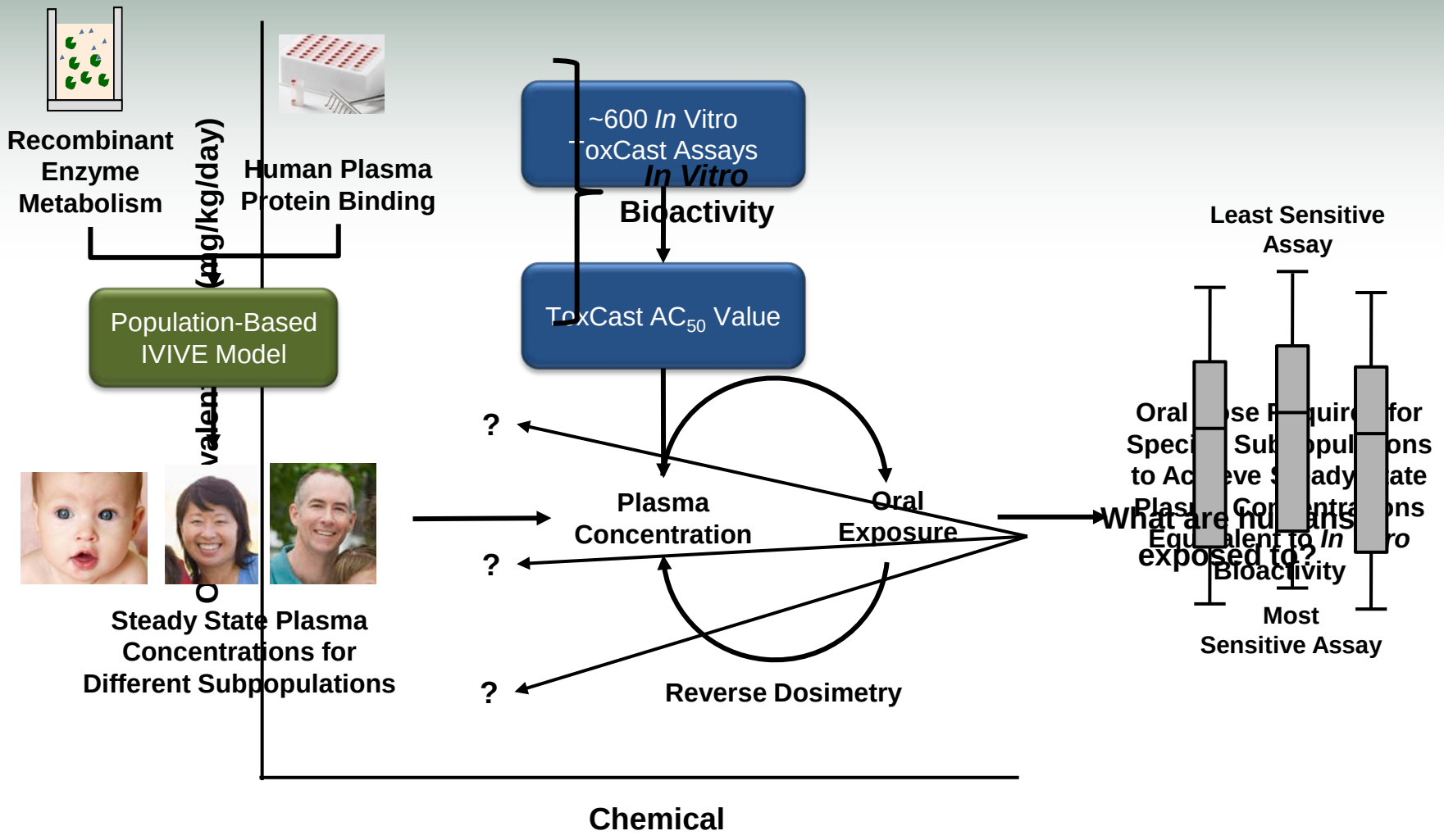
(dimensionless)

Cl_{int} = intrinsic clearance
HLM = human liver microsomes
rCYP = recombinant CYP isoform

Hepatic CYP Isozyme Abundance in Healthy Adults (% of Total)



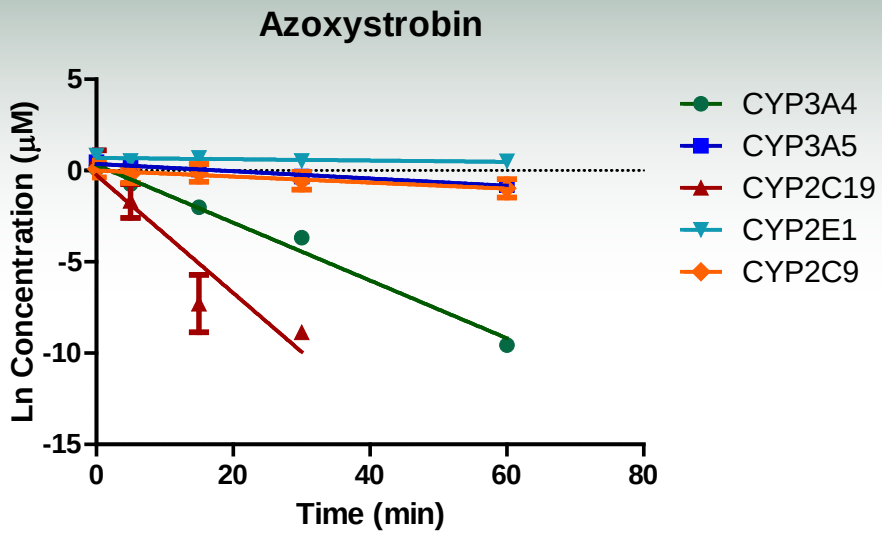
Integrating High-Throughput Pharmacokinetics with the ToxCast *In Vitro* Assays

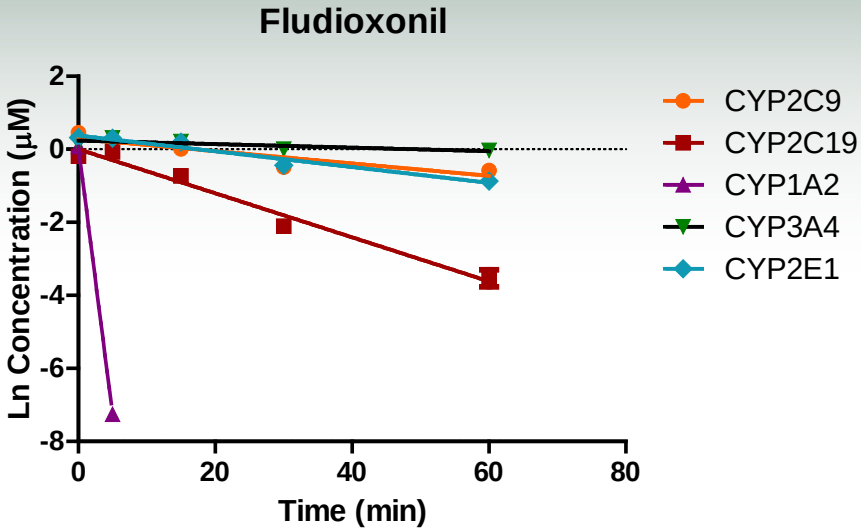


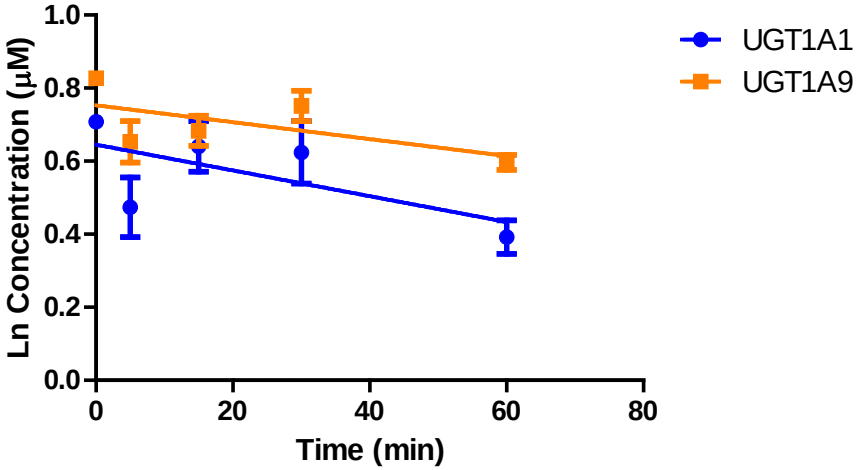
Experimental Design

Test System:	BD Supersomes.
Enzymes:	13 CYPs, 5 UGTs, 2 controls, 1 human liver microsome pool.
Positive Controls:	Suitable substrate for each enzyme, in duplicate.
Chemicals:	9
Negative Controls:	Enzymes lacking cofactors & metabolically inactive supersomes.
Time Points:	60 minute time course; 0 min, 5 min, 15 min, 30 min, 60 min.
Concentrations:	1 µM & 10 µM, in triplicate.

Recombinant Isozyme Clearance Rates

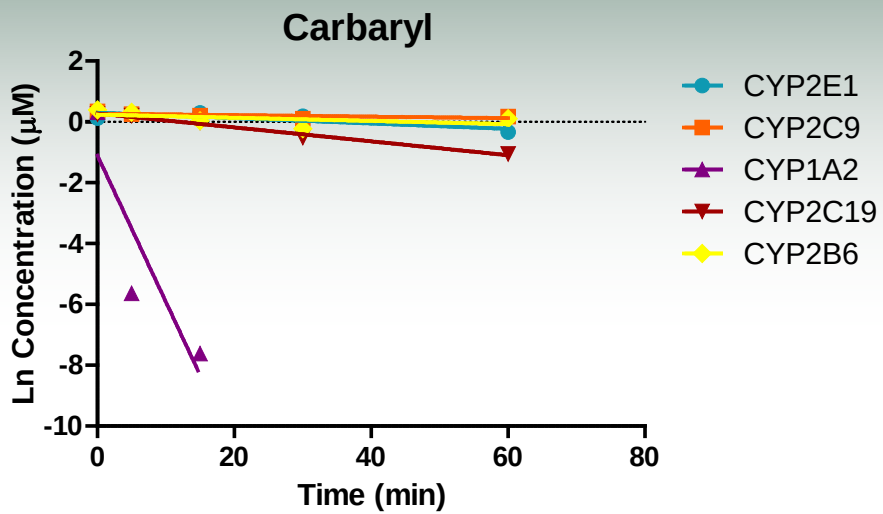


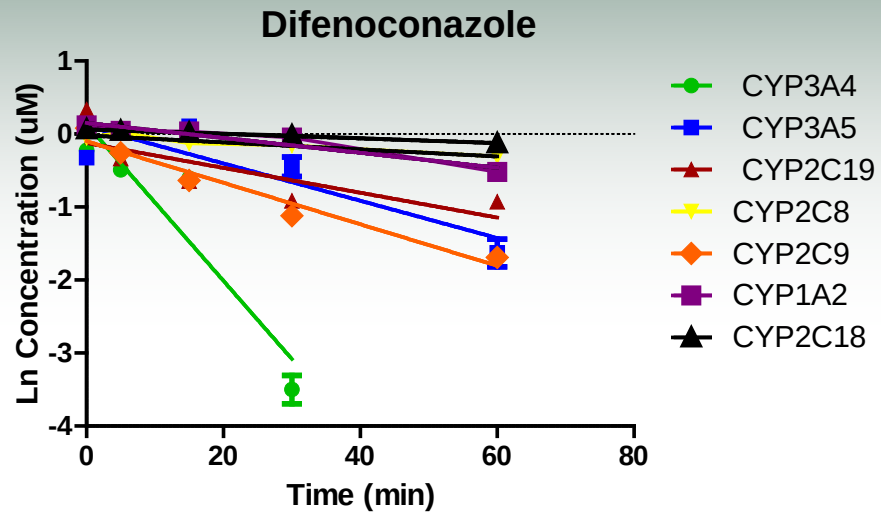




No UGT metabolism detected

Recombinant Isozyme Clearance Rates





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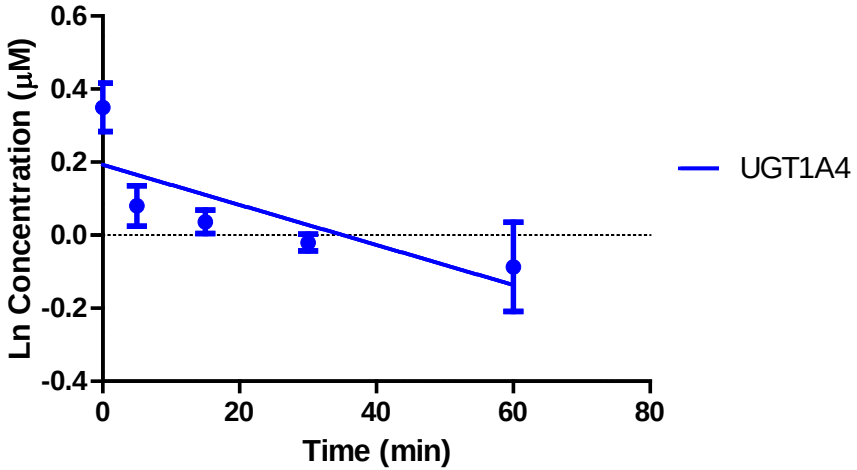
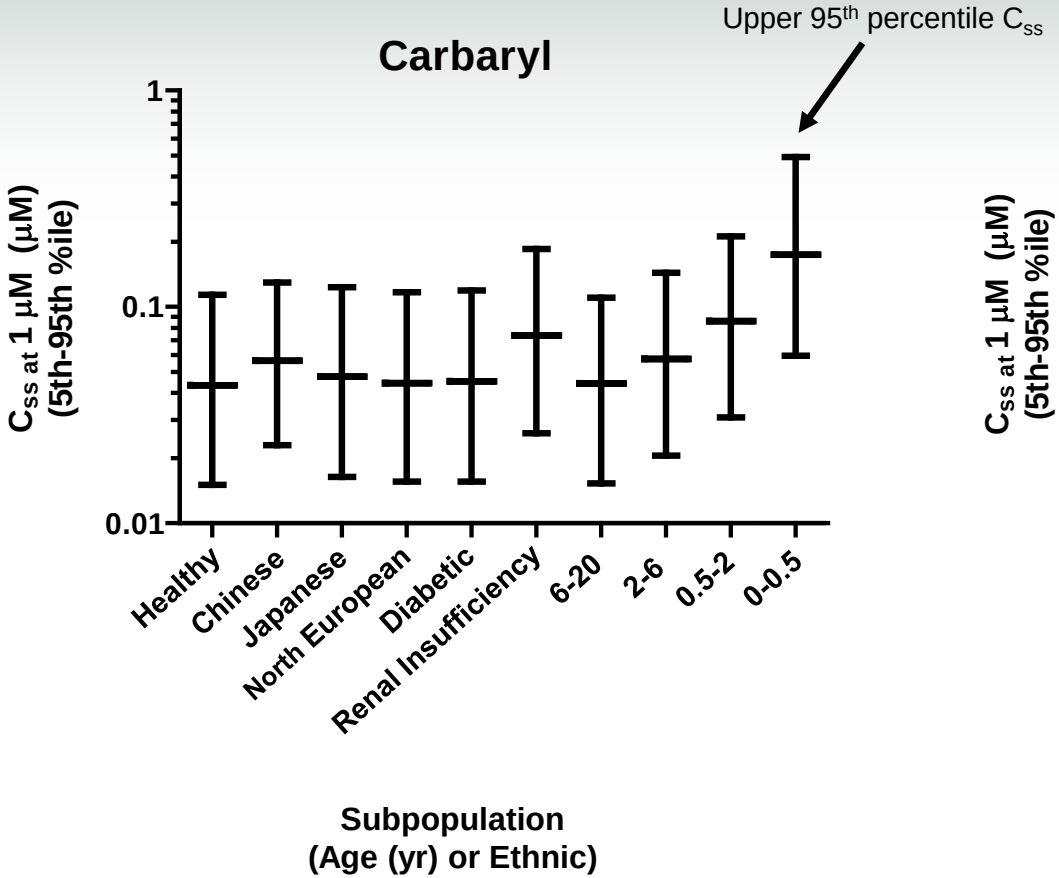
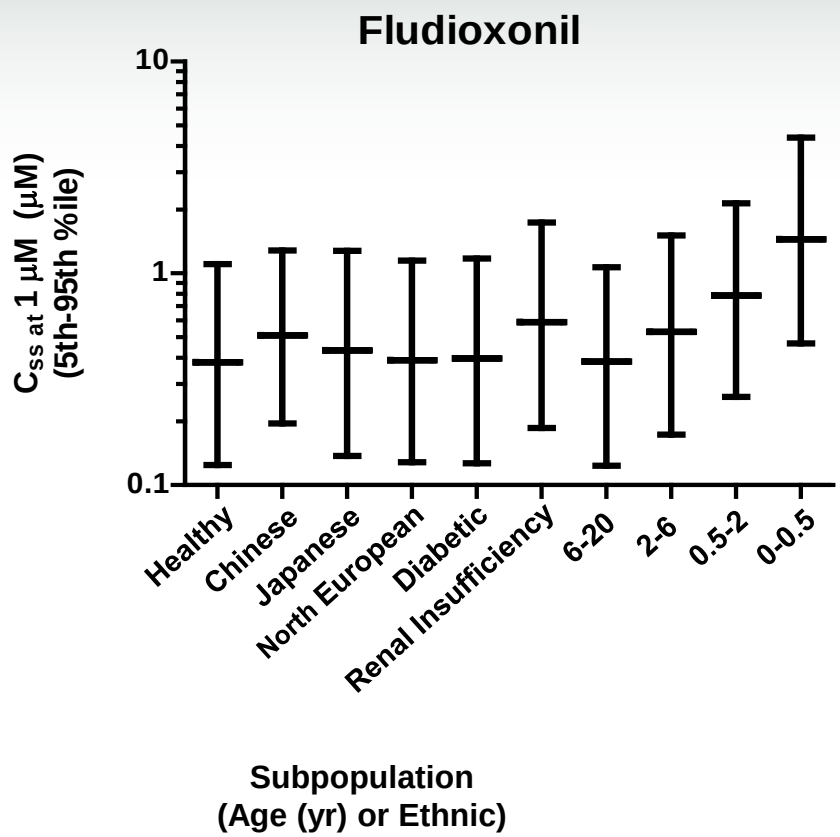


Combining Isozyme Clearance and Abundance Data to Determine Fraction Metabolized

Isozyme	No. Chemicals % fm > 5%	% fm Range	Chemicals with % fm > 5%
CYP1A2	3	0.4 - 91.4	Bensulide, Carbaryl, Fludioxonil
CYP2C9	6	2.1-63.1	Azoxystrobin, Bensulide, Carbaryl, Difenoconazole, Haloperidol, Tebupirimfos
CYP3A4	7	1.0-80.2	Acetochlor, Azoxystrobin, Bensulide, Difenoconazole, Haloperidol, Lovastatin Tebupirimfos
CYP3A5	2	1.4-6.4	Lovastatin, Tebupirimfos
UGT1A1	2	2.6-19.3	Haloperidol, Tebupirimfos
UGT1A4	3	0.1-12.1	Difenoconazole, Haloperidol, Lovastatin

Comparison of C_{ss} Values Derived Across Multiple Subpopulations





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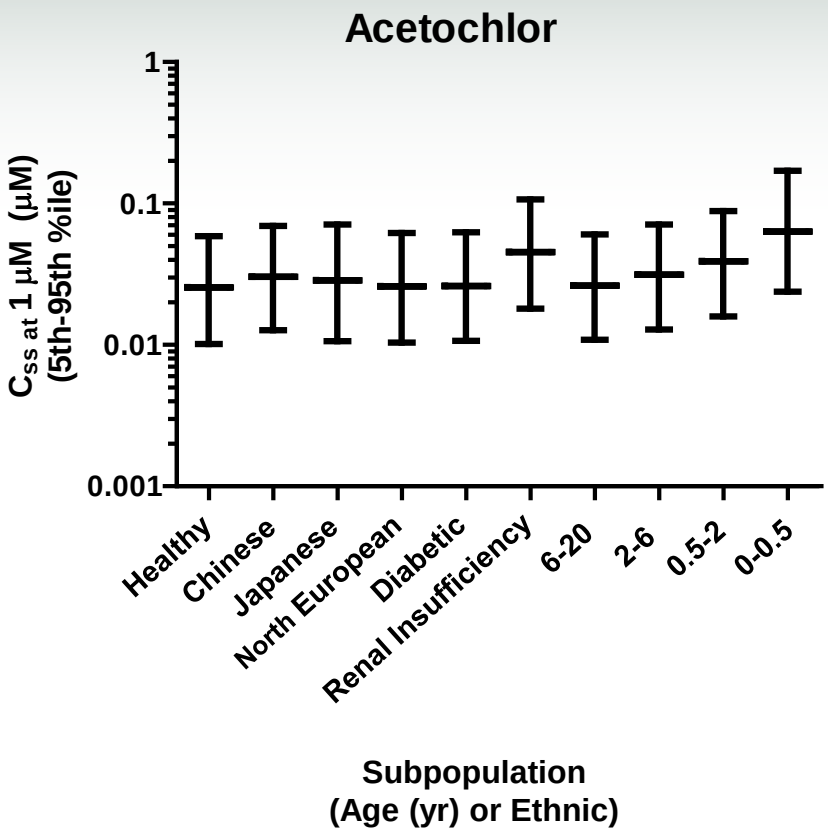
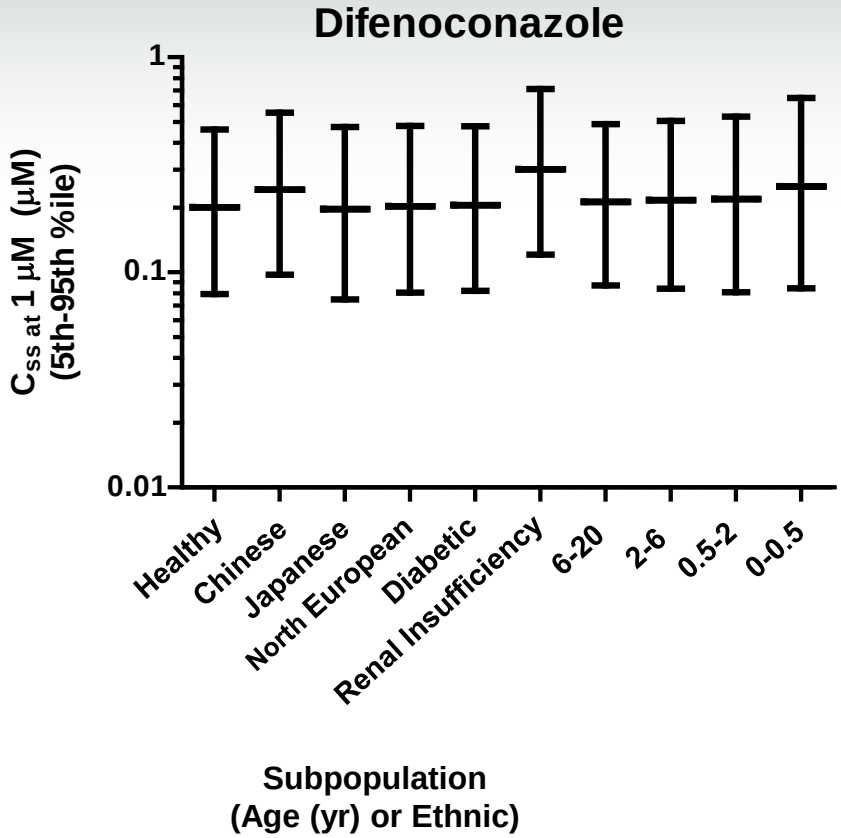




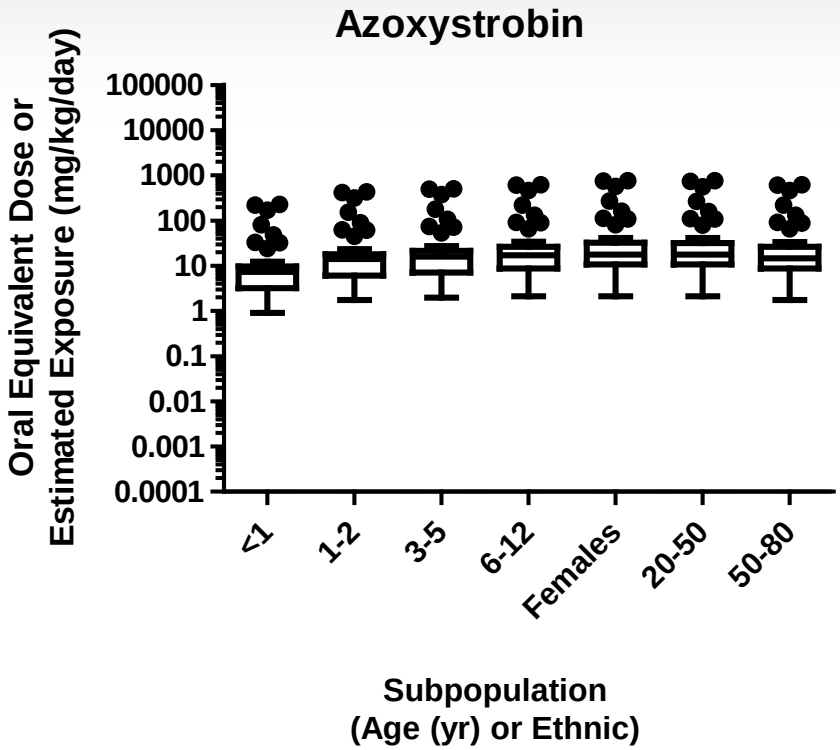
Agreement between *In Vivo* and IVIVE-derived C_{ss} Values using Recombinant CYP-based Clearance Rates

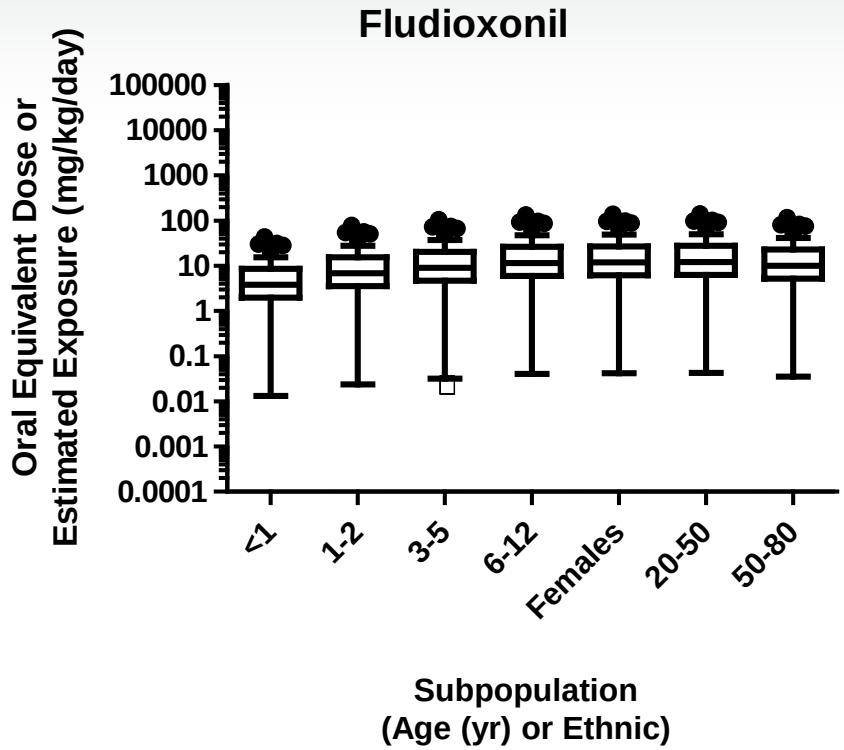
Chemical	*In vivo* PK C_{ss} (μM)	IVIVE C_{ss} (μM)
Carbaryl	0.030	0.046
Haloperidol	0.090-0.126	0.029
Lovastatin	0.004-0.009	0.001

Estimated Chemical-Specific Pharmacokinetic Adjustment Factors

Chemical	Median C_{ss} for Healthy Population	95th Percentile C_{ss} for Most Sensitive	Most Sensitive	Estimated PK-AF	% Contribution of Isozyme Differences to Average PK-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

Matching Oral Equivalent Doses and Exposure Estimates for Subpopulations





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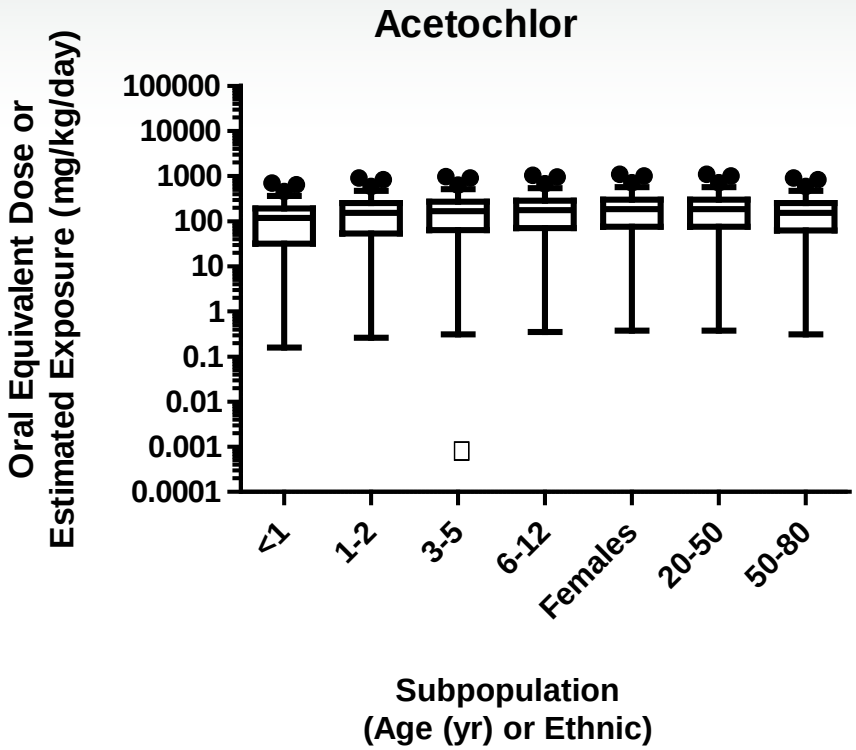

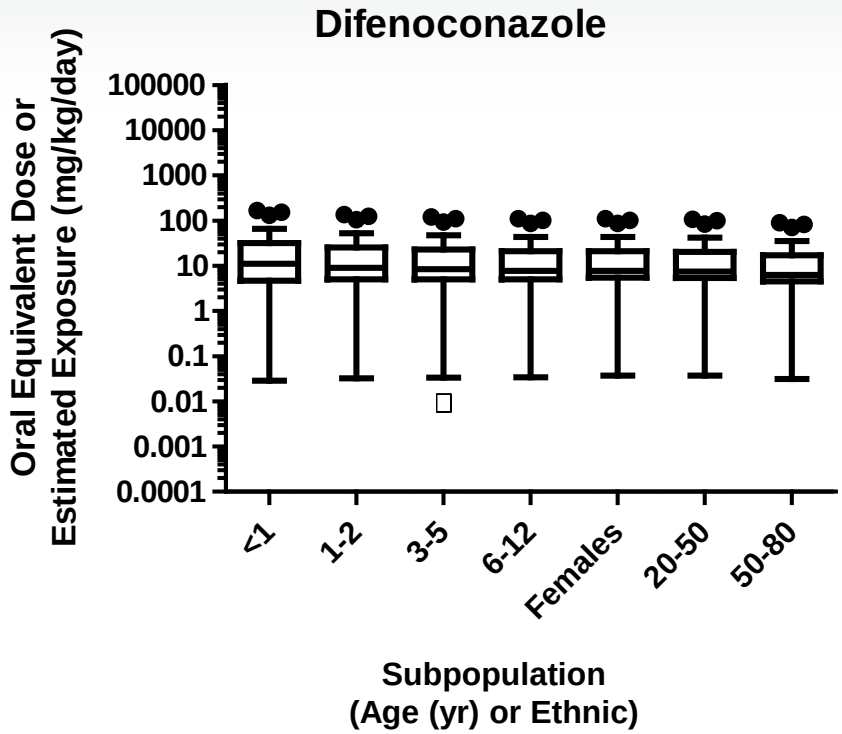

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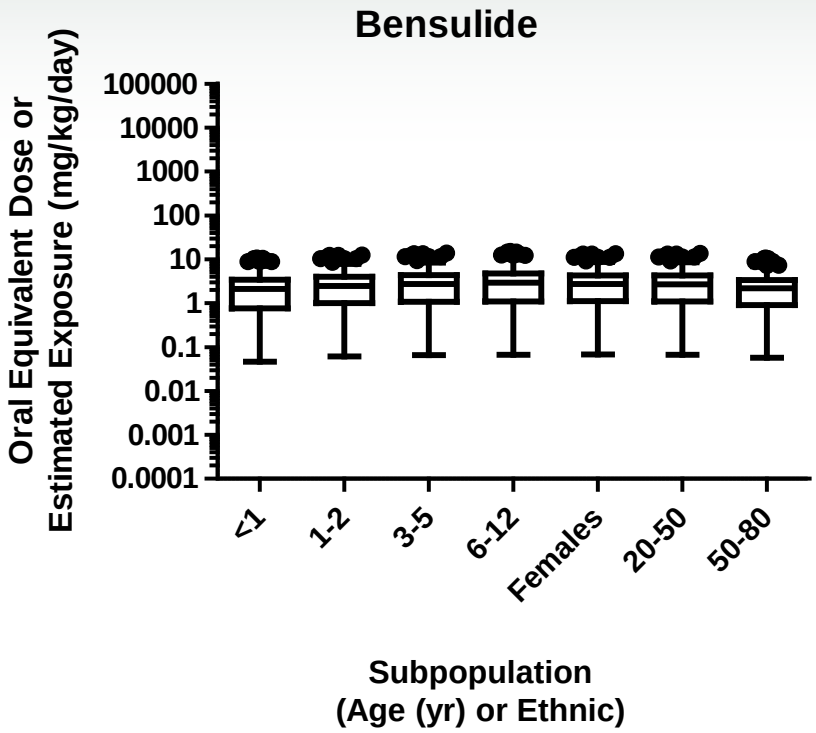

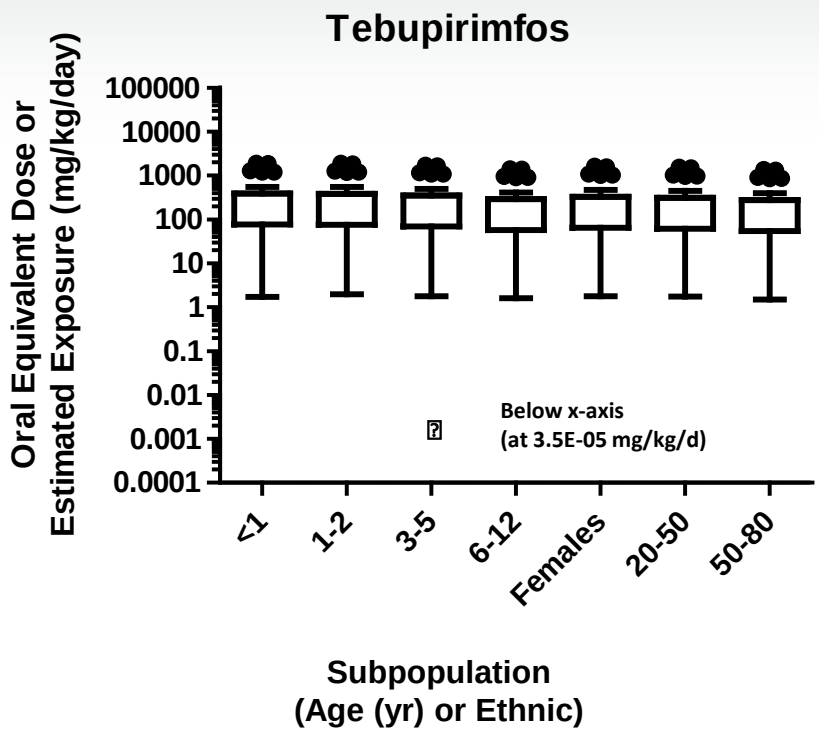

Conclusions

- Demonstrates the feasibility of measuring isozyme-specific clearance rates and using them to capture subpopulation variability for industrial chemicals.
- IVIVE-derived C_{ss} values were in good agreement for C_{ss} values derived from *in vivo* data.
- The pharmacokinetic variability observed when comparing general to the most sensitive population spanned a range of 3 to 11.5-fold.
- The extent of this variability was determined primarily by a chemical's overall clearance rate.
- Subpopulation-based pharmacodynamic differences will also contribute to the variable susceptibilities that may be observed following chemical exposure.

Key Points

- First comprehensive attempt to combine physiologic and PK differences to quantitate variability anticipated between age, ethnic and disease-based populations.
- While the chemical-specific PK adjustment factors routinely exceeded the default 3.2-fold UF assigned for PK-based variability, the adjustment factors for these chemicals were typically within 10-fold (max AF = 11.5).
- When population variability is incorporated with HTS data and exposure information it becomes clear that exposure, rather than hazard, remains a key driver of risk assessment.

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Masoud Jamei (Simcyp)

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