Quantitating population toxicokinetic variability utilizing recombinant enzyme-specific clearance rates

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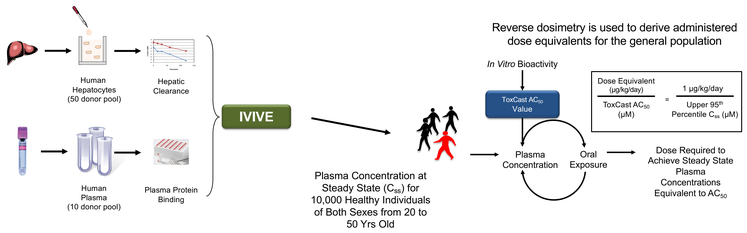
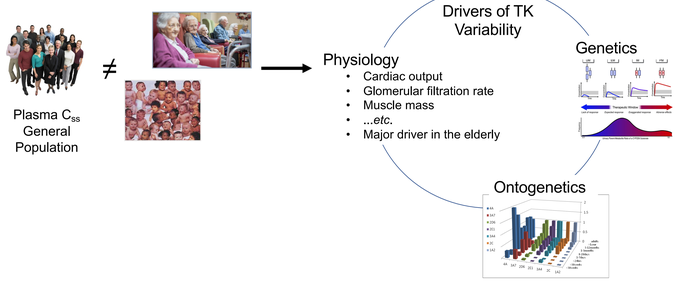
Abstract

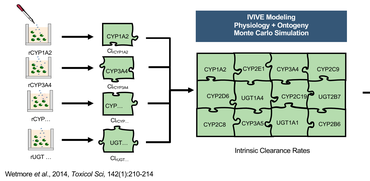
When toxicokinetic (TK) variability occurs across different populations or lifestages, identical external chemical exposures may yield differing systemic concentrations, and may subsequently result in differing health impacts. *In vitro–in vivo* extrapolation (IVIVE) modeling that combines *in vitro* TK data with population-specific physiologic and ontogenetic information during Monte Carlo simulations can be used to predict *in vivo* exposures. A previous proof of concept applied this approach and successfully predicted exposures for drugs for which *in vivo* data were available and demonstrated how interindividual variability could be quantitated. In this follow-up, enzymatic clearance data has been generated for 6 chemicals thus far—ametryn, dimethenamid, fenbuconazole, fenhexamid, glyphosate, and n-butylparaben—across 6 isozymes we previously found to be the predominant contributors to metabolism. The resulting data are being used to further our understanding of whether particular isozyme metabolic profiles may drive greater TK variability. Prediction of steady state concentrations and human-specific TK adjustment factors (HK_{AF}s) thus far show the pediatric lifestage is often the most sensitive, with HK_{AF} values falling above the 3.2 default uncertainty factor typically assigned for TK variability. Sensitivity in the pediatric lifestages decreases with age, coinciding with maturation of the metabolic enzymes. We also noted an inverse relationship between HK_{AF} variability and the number of enzymes with fraction of compound metabolized exceeding 3% for these chemicals. Future work will continue to expand the chemical space analyzed and our assessments of isozyme metabolic profiles to TK variabilities. *This abstract doesn't necessarily reflect the views of the EPA.*

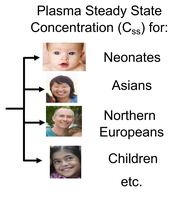
Dosimetry & TK Variability





Approach & Prior Work





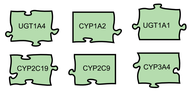
Continuation of this Work

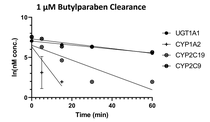
- Expansion of current chemical space with major CYPs & UGTs
- Identify metabolic profiles resulting in greater variability
- Explore lifestage effects more fully

Identify chemicals likely cleared by CYP & UGT panel using ADMET Predictor

Compound	Type
Ametryn	Herbicide
Dimethenamid	Herbicide
Fenbuconazole	Triazole fungicide
Fenhexamid	Fungicide
Glyphosate	Herbicide
Butylparaben	Antimicrobial used in cosmetics, medications, flavoring

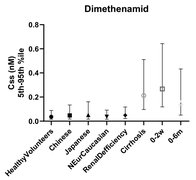
Screen across panel of CYPs and UGTs





Determine clearance rates for chemicals by individual enzymes
- Loss of parent
- Analyzed using UPLC-MS/MS

C_{ss} Predictions
- Monte Carlo simulation using SimCyp
- Chemical-specific:
  - physicochemical info
  - rCYP clearance rates
- Trial Size: N=1000
- Dose: 1 µg/kg/day
- Several populations / lifestages (using libraries pre-parameterized with relevant physiologic information)



Calculate Human Toxicokinetic Adjustment Factor

$$HK_{AF} = \frac{95^{th}\ percentile\ C_{ss}\ for\ the\ most\ sensitive\ population}{median\ of\ healthy\ population}$$

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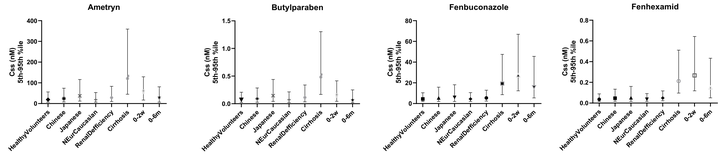
Preliminary Results

Enzyme-specific Clearance Rates (µl/min/pmol p450 or mg protein) (0.1µM, 1µM)

Chemical	CYP1A2		CYP2C9		CYP2C19		CYP3A4		UGT1A1		UGT1A4	
	0.1µM	1µM	0.1µM	1µM	0.1µM	1µM	0.1µM	1µM	0.1µM	1µM	0.1µM	1µM
Ametryn*	0	0	0	0	0.631	0.708	0	0	0	0	0	0
Dimethenamid	0	0	0.063	0.057	0	0	3.28	4.00	0	0	0	0
Fenbuconazole	0	0.076	0	0	0.125	0	1.17	0	0	0	0	0
Fenhexamid	0	0	0.0470	0	1.18	0.724	0	0	116.539	117.276	0	0
Glyphosate	0	0	0	0	0	0	0	0	0	0	0	0
Butylparaben*	6.727	8.559	0.237	0.150	2.232	1.822	0	0	49.284	42.744	0	8.198

*Ametryn, & UGT1A1 & UGT1A4 for Butylparaben were run at 1 & 10µM

The early pediatric lifestage and cirrhosis patients are generally the most sensitive populations across this set of chemicals.



Human TK adjustment factors (HK_{AF}s) for the most vulnerable subgroups fall above the default uncertainty factor of 3.2.

- Ametryn=18.83 for patients with severe cirrhosis
- Butylparaben=17.71 for patients with severe cirrhosis
- Dimethenamid=17.54 for 0-2-week olds
- Fenbuconazole= 15.07 for 0-2-week olds
- Fenhexamid=8.98 for 0-2-week olds

There is an inverse relationship between HK_{AF} variability and the number of enzymes by which a compound is metabolized.

Summary and Future Directions

- Data generation is currently underway for 12 chemicals; with clearance data for 6 described here.

Future work will:
- More closely define variability ranges within first 6 months of life;
- Examine the relationship between fraction of compound metabolized and population variability;
- Seek out trends and metabolic profiles that may yield greater differences across lifestages;
- Assess clearance by additional enzymes;
- Compare C_{ss} values to exposure estimates for these chemicals to help inform regulatory decision-making regarding uncertainty factors.

References

(1) Wetmore B. A., Allen B., Clewell H. J. 3rd, Parker T., Wambaugh J. F., Almond L. M., Sochaski M. A., Thomas R. S. (2014). Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing. *Toxicol. Sci.* 142, 210–224.
(2) Cohen Hubal, E.A., de Wet, T., Du Toit, L., Firestone, M.P., Ruchirawat, M., van Engelen, J., and Vickers, C. (2014). Identifying important life stages for monitoring and assessing risks from exposures to environmental contaminants: Results of a World Health Organization Review. Regul. Toxicol. Pharmacol. 69: 113-124.
(3) Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S., and Goble, R. (2002). Evaluation of Child/Adult Pharmacokinetic Differences from a Database Derived from the Therapeutic Drug Literature. Toxicol. Sci. 66: 185-200.

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