Quantitating Population Toxicokinetic Variability Utilizing Recombinant Enzyme-Specific Clearance Rates

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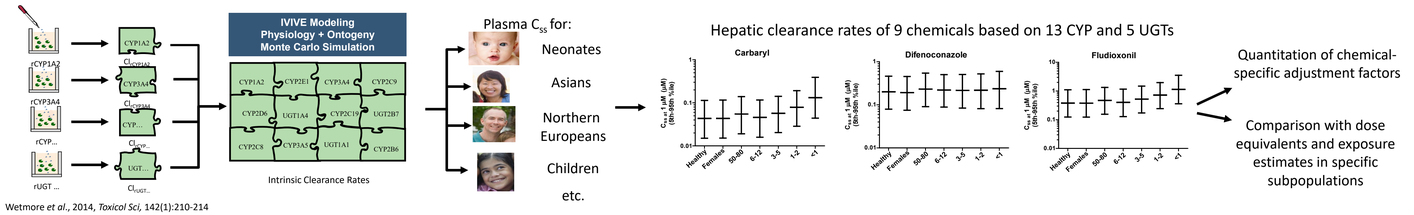
Abstract

High throughput screening (HTS) methods are becoming the norm in toxicological testing. These HTS methods require incorporation of dosimetry studies to estimate steady state concentrations of chemicals in human subjects. However, dosimetry methods are applicable to the general population and miss potential variability that may occur in different subpopulations and lifestages. To account for such variability and provide safety in risk estimates, an uncertainty factor is incorporated. It is unclear, though, how appropriate this uncertainty factor is for particular vulnerable subpopulations. Studies to better understand population variability are underway and are looking at which populations are most sensitive, the extent of variability, and contributors to variability. To better understand contributors to population variability and inform HTS assays, previous work from our lab looked at clearance rates of chemicals by different cytochrome P450's (CYPs) and uridine diphosphoglucuronosyl transferases (UGTs). Using these clearance rates, steady state concentrations were estimated and used to inform chemical-specific adjustment factors and compared with oral equivalents and exposure estimates in specific subpopulations. We are now continuing this work, adapting it for a more high-throughput screening method and broadening the chemical space assessed.

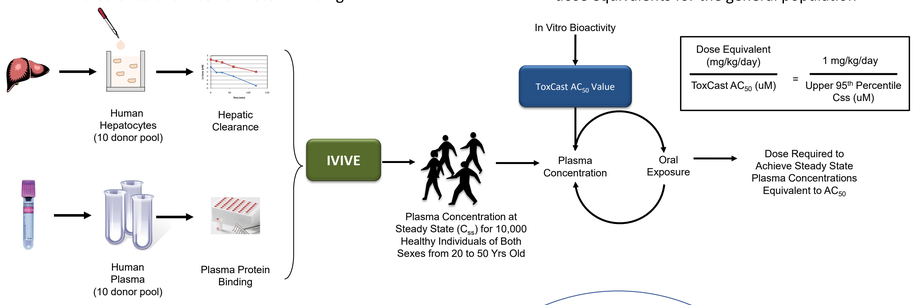
Experimental Design & Prior Work



Wetmore *et al.*, 2014, *Toxicol Sci,* 142(1):210-214

Background

Human dosimetry estimates are derived based on Hepatic Clearance and Plasma Protein Binding



Reverse dosimetry is used to derive administered dose equivalents for the general population

$$\frac{\text{Dose Equivalent (mg/kg/day)}}{\text{ToxCast } AC_{50} \text{ (uM)}} = \frac{1 \text{ mg/kg/day}}{\text{Upper 95th Percentile Css (uM)}}$$

However…

Drivers of TK Variability

Physiology
- Cardiac output
- Glomerular filtration rate
- Muscle mass, water content
- …*etc.*
- Major driver in the elderly

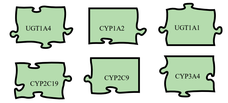
Genetics

Ontogenetics

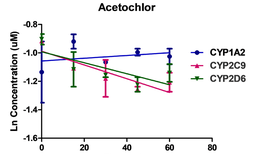
Continuation of this Work

- Expansion of current chemical space with experimental CYP/UGT data
- Identify metabolic profiles resulting in greater variability
- Explore lifestage effects more fully

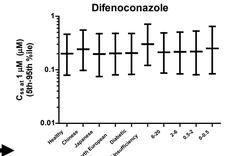
Identify chemicals likely cleared by CYP & UGT panel using ADMET Predictor



Screen across panel of CYPs and UGTs

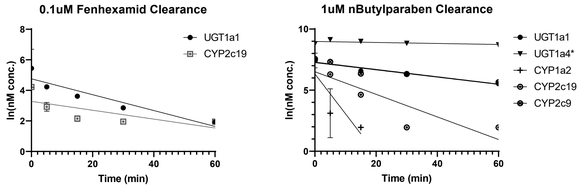


Determine clearance rates for chemicals by individual enzymes



Feed predictions into SimCyp to determine C_{ss} for individual populations

Preliminary Results



Enzyme-specific Clearance Rates (ul/min/pmol p450 or mg protein) (0.1uM, 1uM)						
Chemical	**CYP1a2**	**CYP2c9**	**CYP2c19**	**CYP3a4**	**UGT1a1**	**UGT1a4**
Dimethenamid	0	NT	2.43; 3.29	NT	0	0
Fenbuconazole	0; 0.0760	NT	0.125; 0	NT	0	0
Fenhexamid	0	NT	0.291; 0.724	NT	104; 117	0
nButylparaben	6.73; 3.27	0.244; 0.150	1.08; 0.924	NT	59.3; 42.7*	0; 8.20*

NT- not yet tested
*UGT1a1 & UGT1a4 were run at 1 & 10uM

Summary & Future Directions

- Data generation is currently underway for 12 chemicals; with clearance data for 4 described here.

Future work will:

- Incorporate experimental data into IVIVE approach to predict internal Css and quantitate differences across different populations/lifestages;
- More closely define variability ranges within first 6 months of life;
- Seek out trends and metabolic profiles that may yield greater differences across lifestages.

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.