UTILIZING ISOZYME-SPECIFIC CLEARANCE RATES TO INFORM POPULATION TOXICOKINETIC VARIABILITY

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POPULATION TOXICOKINETIC (TK) VARIABILITY

Identical exposures may lead to differing in vivo concentrations and health impacts

Body burden of general population

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Body burden of at risk populations

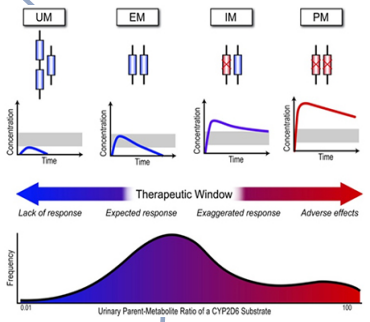
WHY?

DRIVERS OF TK VARIABILITY

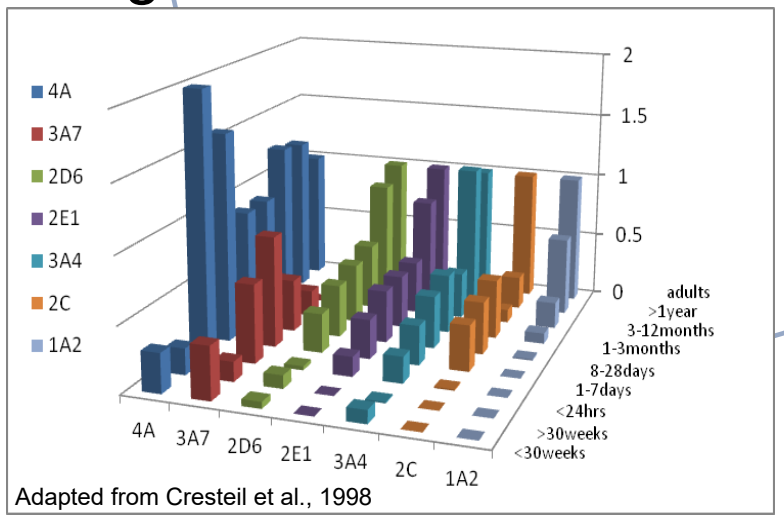
Physiology

- Variation in ADME processes
- Major driver in the elderly

Genetics



Ontogenetics

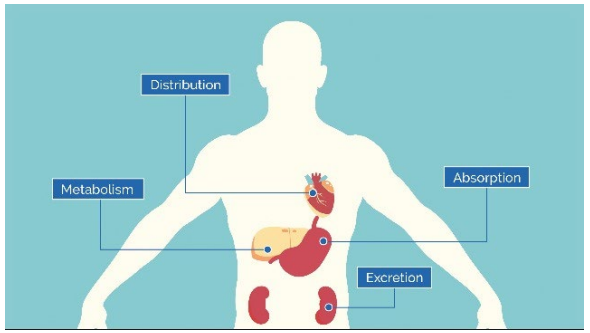


Adapted from Cresteil et al., 1998

Contributors to Variability	Effect Window	Extent of Effect	Frequency
Physiologic (e.g., tissue weights, blood flow rates)	All lifestages; greatest early & late	Moderate	All populations & lifestages
Ontogenetic (e.g., differing abundances in enzymes, transporters, etc.)	Early lifestages	Can be significant	All within relevant lifestages
Genetic (e.g., functional differences in enzymes, transporters)	All life stages	Depends on polymorphism	0-10% of population
Exposomic (e.g., co-exposures, lifestyle, microbiome)	Throughout life	Unkown	Unknown

DRIVERS OF TK VARIABILITY: PHYSIOLOGY

- Cardiac output
- Glomerular filtration rate
- Muscle mass, water content
- Enzymatic ½ life



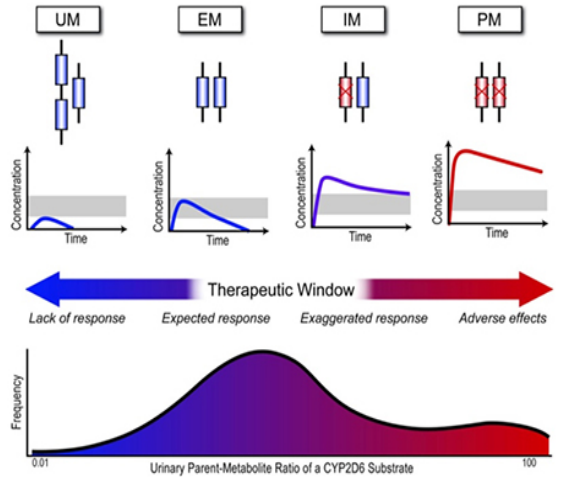
- Volume of distribution
- Chemical half life

DRIVERS OF TK VARIABILITY: GENETICS

- Insights from genomics
- CYP2D6
  - Drug metabolism
  - >100 variants
  - 100+X functional variability

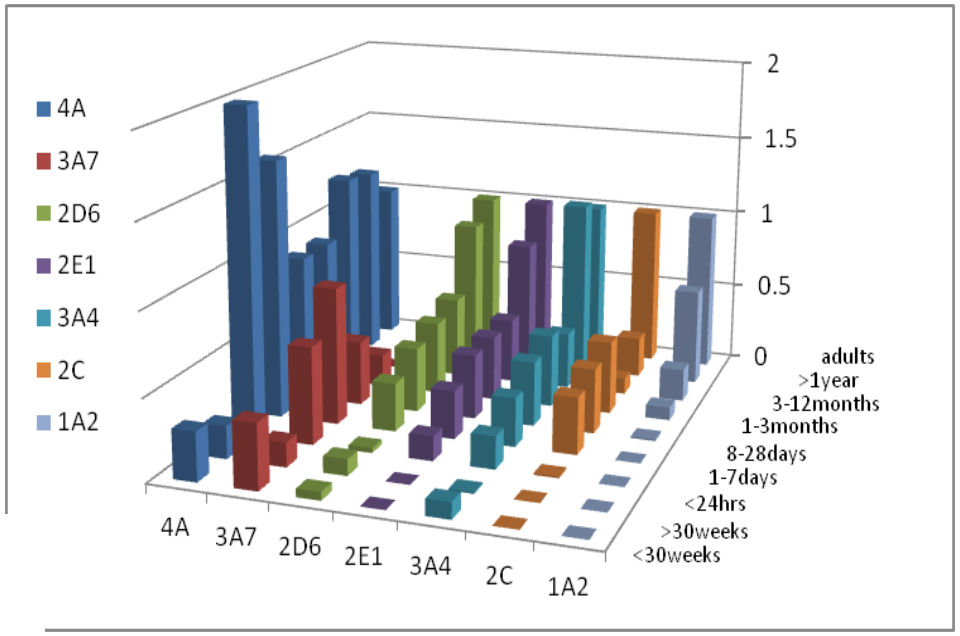
CYP2D6

Copy #	Metabolizer Group	% Distribution
2+	Ultrarapid	5
2	Extensive	70
1	Intermediate	15
0	Poor	10



DRIVERS OF TK VARIABILITY: ONTOGENETICS

- Liver bank studies
- Differences in abundances
  - Binding affinities
  - Absorption
- Functional overlap



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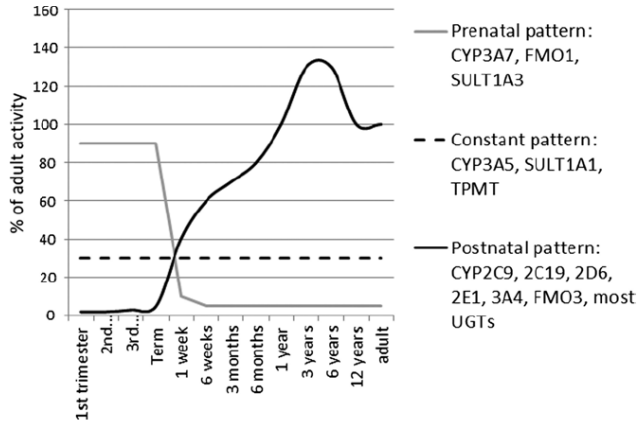
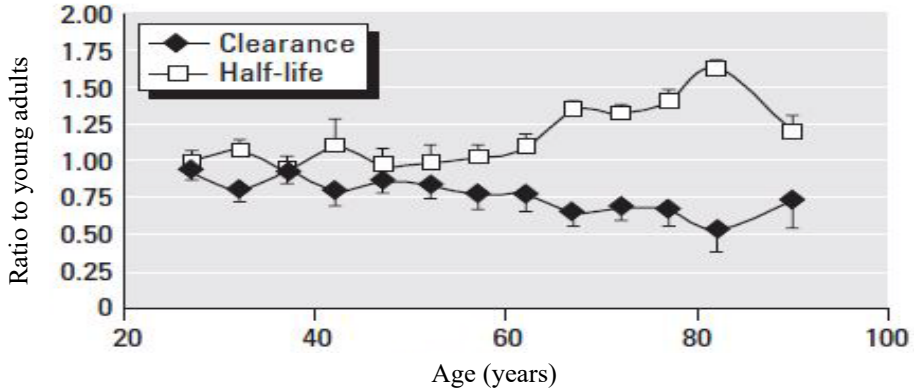


DRIVERS OF TK VARIABILITY IN CHILDREN

Developmental Feature	Relevant Lifestage	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 functionality	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

DRIVERS OF TK VARIABILITY IN THE ELDERLY

- < Cardiac outputs, tissue blood flow (hepatic – 25% ↓)
- < Muscle mass, body water (up to 25% ↓)
- > Lipid content (↑ Vd; longer T1/2, lipophilic compounds)
- < Plasma protein binding (15-25% ↓; higher free drug conc.)
- < Renal clearance, glomerular filtration rate
- < Hepatic clearance (↓ liver size, P450 content, bile flow, blood flow)



Hattis & Russ, 2003

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

ADDITIONAL CONSIDERATIONS FOR TK VARIABILITY

- Exposomic- diet, exercise, drugs
- Mixtures
- Contributors working in parallel
  - Overlap, may obviate contribution

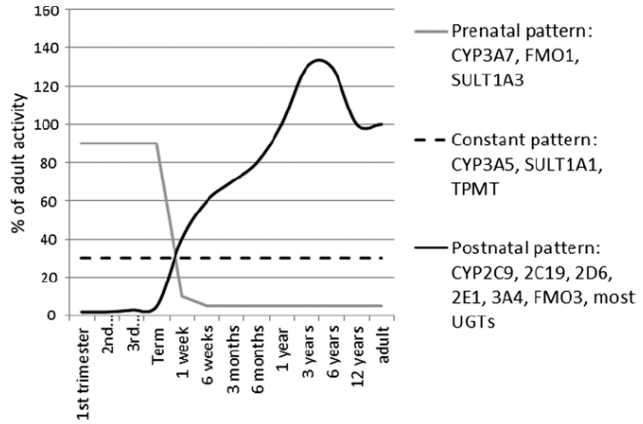
IMPLEMENTATION OF TK VARIABILITY

- Dearth of data
- Rarely incorporated into tox studies
- Clinical studies typically on Caucasian Healthy Volunteers
- Need for systematic approach

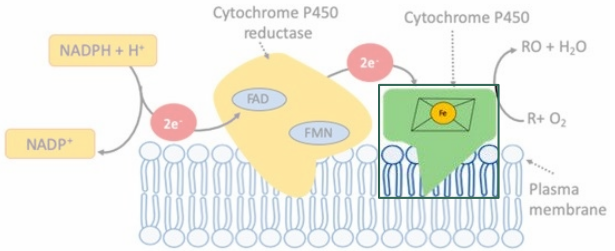
USING RECOMBINANT ISOZYMES TO STUDY TOXICOKINETIC VARIABILITY



- Informs population & lifestage variability

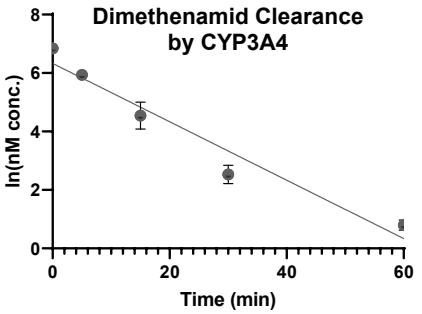


Recombinant Isozyme, i.e. Cytochrome P450 1A2



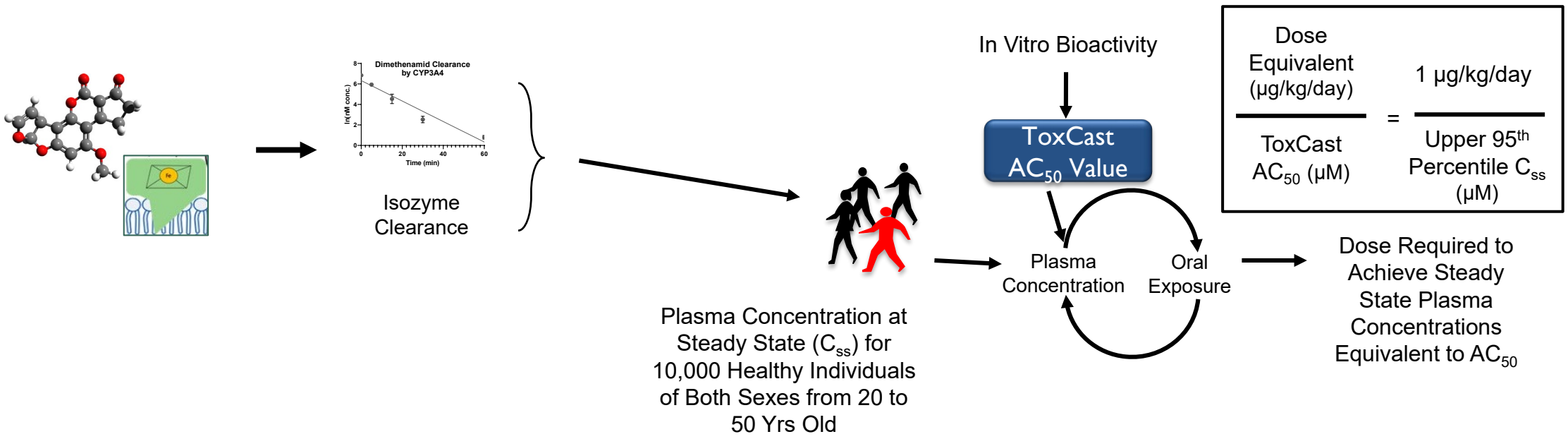
R=Compound of Interest

Substrate depletion: monitor loss of parent compound



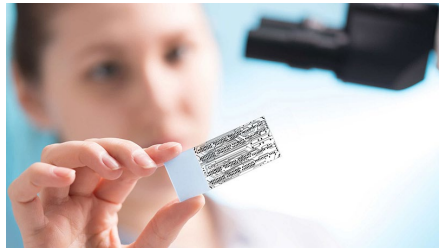
TRANSLATING CLEARANCE RATES INTO HUMAN PLASMA STEADY STATE CONCENTRATIONS

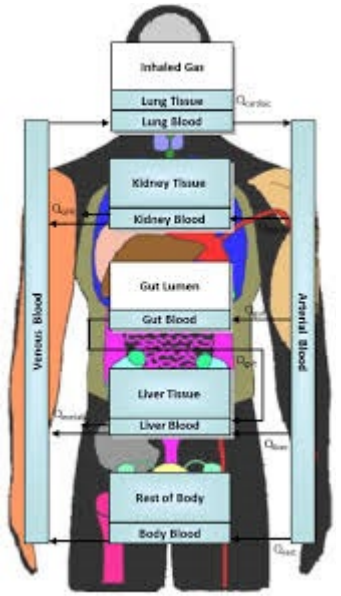
- *In vitro–in vivo* extrapolation (IVIVE) combines *in vitro* TK data with population-specific physiologic and ontogenetic information to predict *in vivo* systemic exposure
- "Reverse dosimetry" used to derive dose equivalents



NEW APPROACH METHODOLOGIES (NAMS)

- Any non-mammalian approach that can inform risk assessment & characterization of chemical hazard
- Mechanism of Action (MOA), Adverse Outcome Pathways (AOPs)
- Inform prioritization
- Modeling

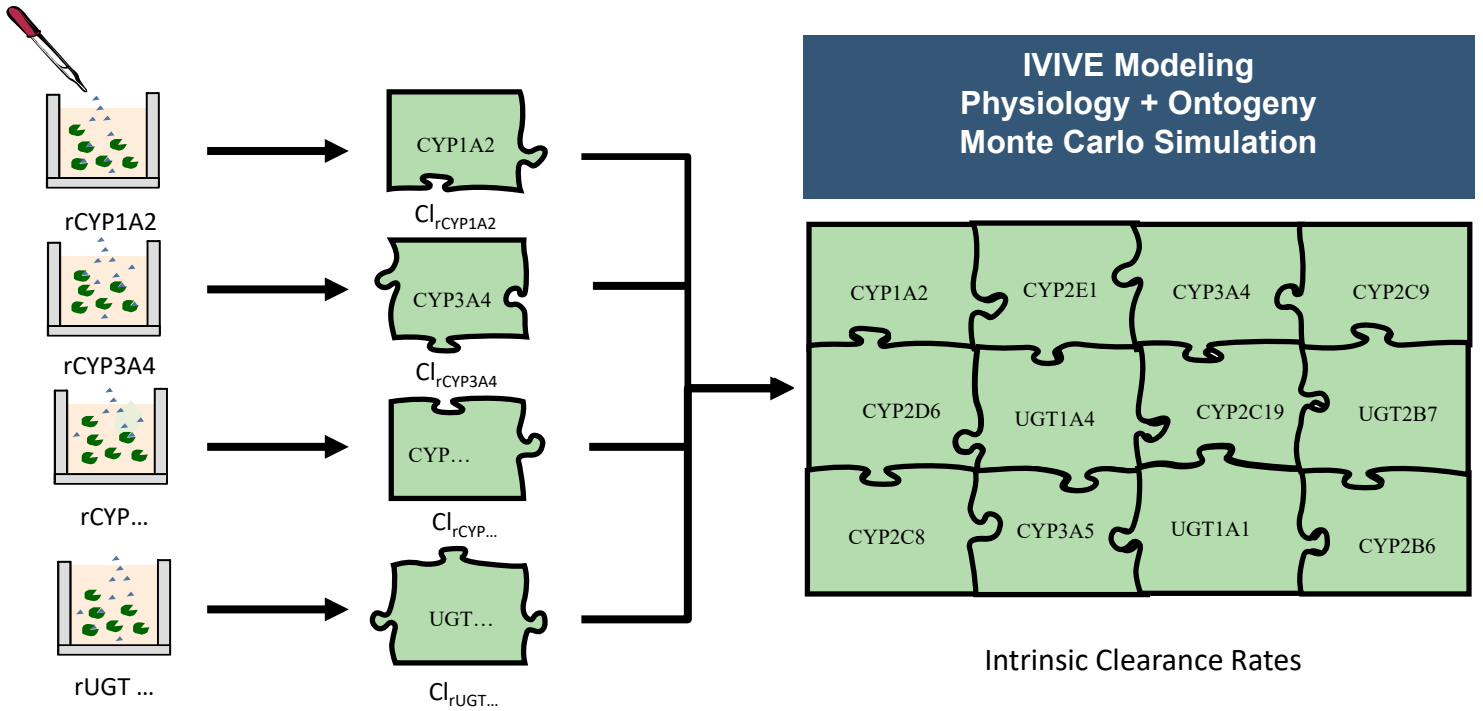




PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING

- Interspecies
- Clinical
  - Dosing, especially pediatric
  - Need for clinical trials
- US FDA
- US EPA
  - httk, httk-pop
  - 80,000 registered chemicals, 30,000 in routine use

PRIOR WORK (WETMORE ET AL., 2014, *TOXICOL SCI*)



Plasma Steady State Concentration (C_{ss}) for:

Neonates

Asians

Northern Europeans

Children

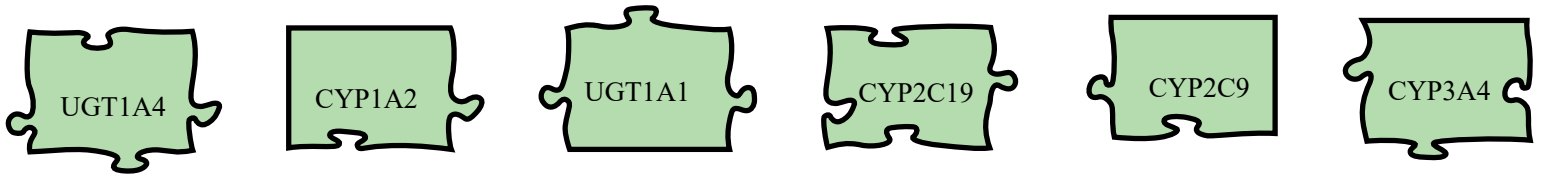
etc.

EXTENSION OF THIS WORK

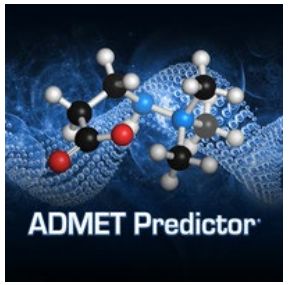
- Higher throughput
- Expanded chemical space
- Examining trends, profiles, lifestage effects

EXTENSION OF THIS WORK

- Higher throughput
  - Major CYPs & UGTs



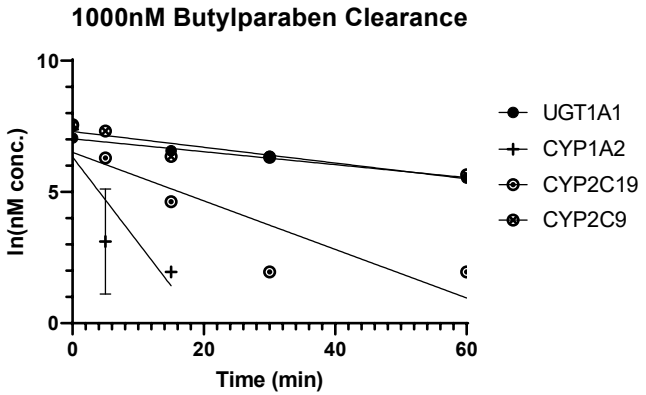
- Expanded chemical space
  - Identify chemicals likely cleared by enzyme panel
  - Elevated exposure
  - Prioritize child-care products



- Examining trends, profiles, lifestage effects

GENERATION OF ISOZYME-SPECIFIC CLEARANCE RATES

- Clearance rates generated for 6 compounds; remaining 6 in progress

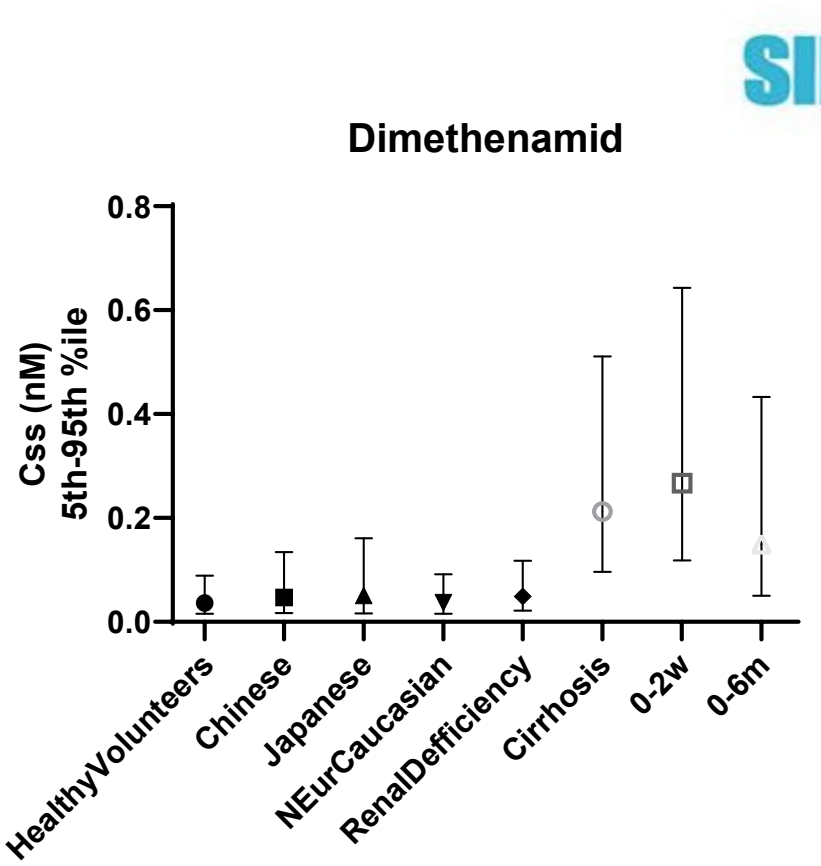


Chemical	CYP1A2	CYP2C9	CYP2C19	CYP3A4	UGT1A1	UGT1A4
Ametryn	X		X			
Butylparaben	X	X	X		X	
Dimethenamid				X		
Fenbuconazole				X		
Fenhexamid		X	X		X	
Glyphosate						
Piperonyl Butoxide						
Diethylhexyl Phthalate						
2-phenoxyethanol						
Phenol						
Styrene						
Propranolol						

TBD

PREDICTION OF C_{SS} FOR SPECIFIC SUBPOPULATIONS

- Monte carlo simulations run using SimCyp (Certara) software
- Population parameters
  - N=1000
  - 1 µg/kg/day
  - *20-50 y*
  - 50% F
- Using predicted
  - Isozyme-specific clearance rates
  - Fraction unbound
  - f_{umic}
  - etc. physicochemical properties



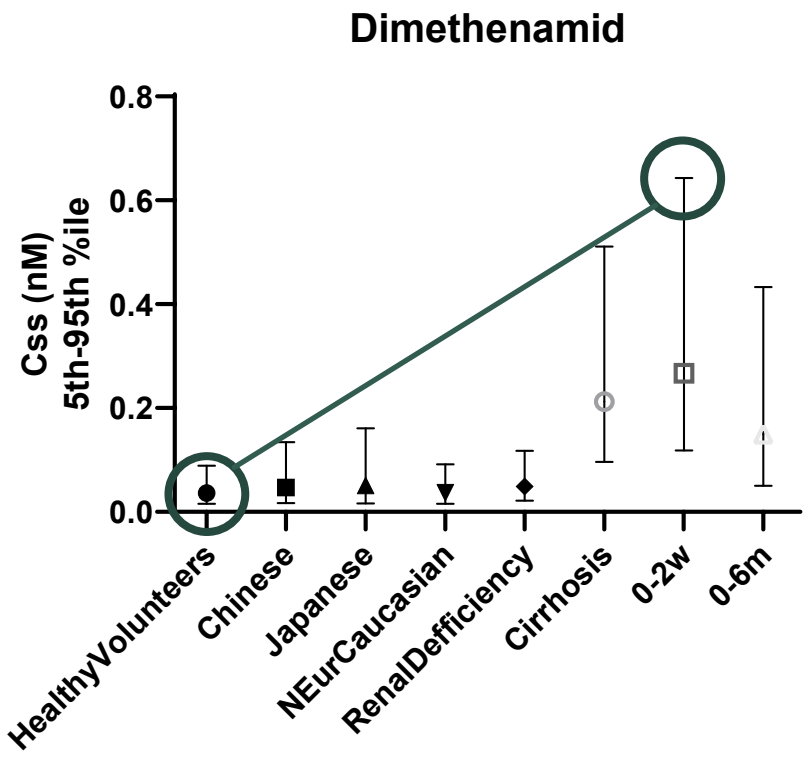
UNCERTAINTY FACTORS (UF) IN RISK ASSESSMENT

- Applied to benchmark dose (BMD), no adverse effect level (NOAEL) to derive acceptable intake
- Typically 100X
  - 10X interspecies variability
  - 10X intraspecies variability
    - 3.2X TK & 3.2X toxicodynamic
- Chemical specific adjustment factors

CALCULATION OF HUMAN TK ADJUSTMENT FACTORS (HK_{AF}) TO ASSESS POPULATION VARIABILITY

- Population variability consists of TK & toxicodynamic variability

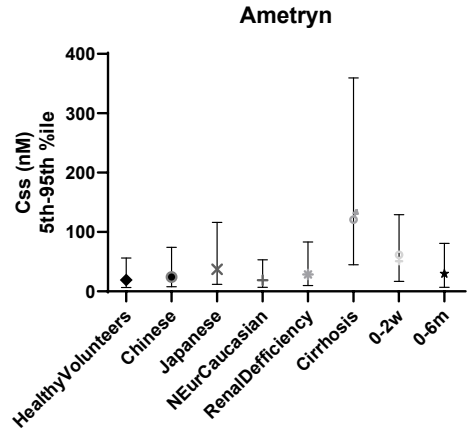
$$HK_{AF} = \frac{95th\ percentile\ Css\ for\ most\ sensitive\ population}{median\ of\ healthy\ population}$$

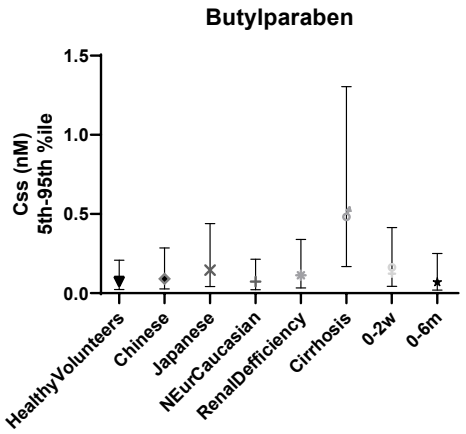


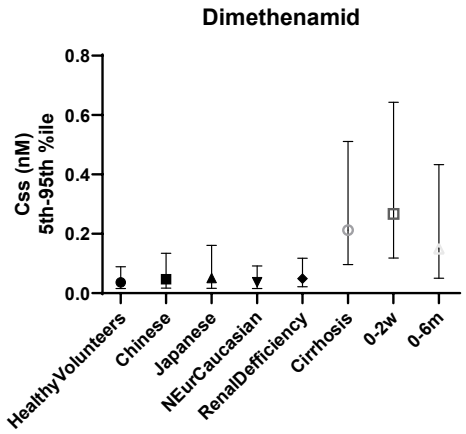
PRELIMINARY RESULTS

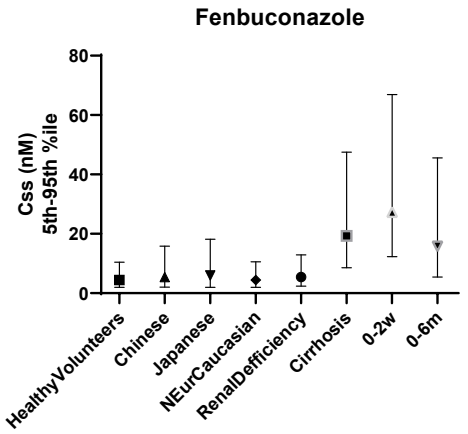
C_{SS} OF SPECIFIC SUBPOPULATIONS

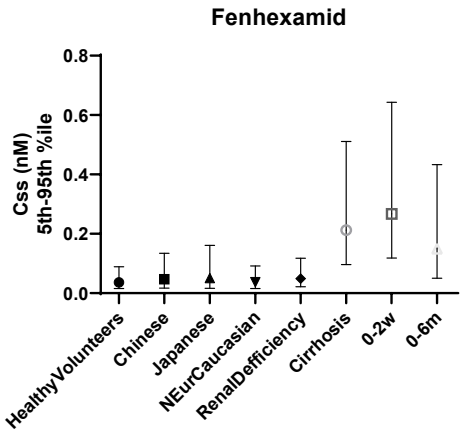
The early pediatric lifestage and cirrhosis patients are generally the most sensitive populations





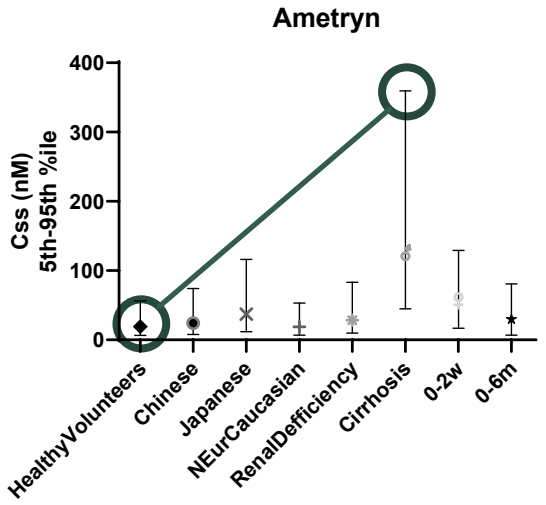






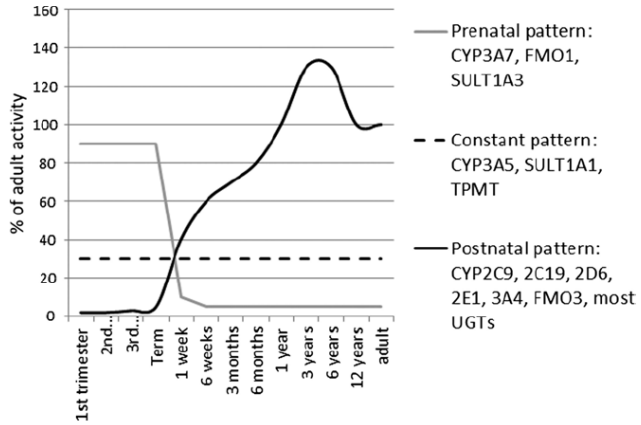
$HK_{AF}S$

- HK_{AF}s for the most vulnerable subgroups fall above the default uncertainty factor of 3.2
  - Ametryn=18.8 for patients with severe cirrhosis
  - Butylparaben=17.7 for patients with severe cirrhosis
  - Dimethenamid=17.5 for 0-2 week olds
  - Fenbuconazole=15.1 for 0-2 week olds
  - Fenhexamid=9.0 for 0-2 week olds



NEXT STEPS

- Examine trends that impact population variability
  - Chemical properties
  - Metabolic profiles
- Mapping out of neonatal variability
- Exposure estimates
  - Population adjusted dose (PAD)
  - Adverse exposure ratios



SUMMARY & FUTURE DIRECTIONS

- Responses to chemicals vary due to population TK variability
- Need for approaches to assess TK variability
- Need for incorporation into risk assessment
- Data generation is currently underway for 12 chemicals; with clearance data for 6 described here.
- The early pediatric lifestage and cirrhosis patients are generally the most vulnerable subpopulations.

Future work will:

- More closely define variability ranges within first 6 months of life;
- Examine trends that may contribute to population variability;
- Compare C_{ss} values to exposure estimates for these chemicals to help inform regulatory decision-making regarding uncertainty factors.

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QUESTIONS?

DRIVERS OF TOXICOKINETIC (TK) VARIABILITY

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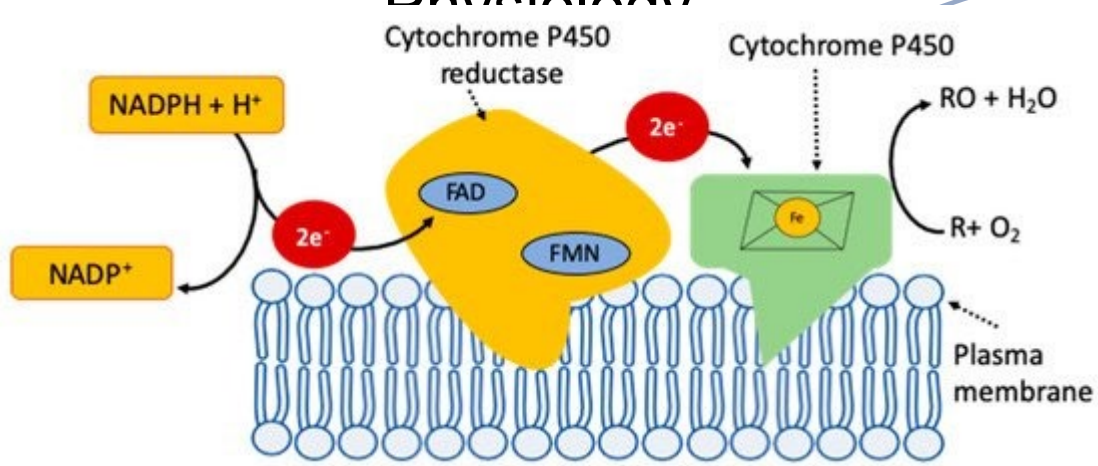
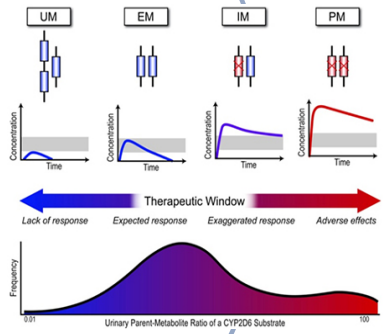
- Informs population & lifesta

Physiology



Genetics



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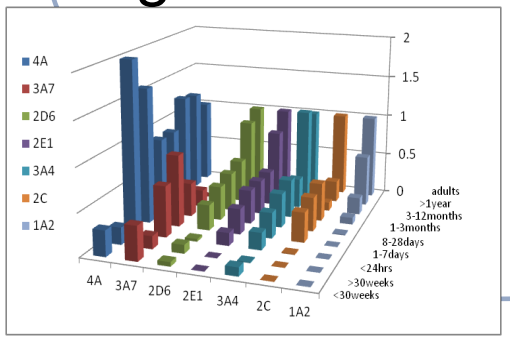

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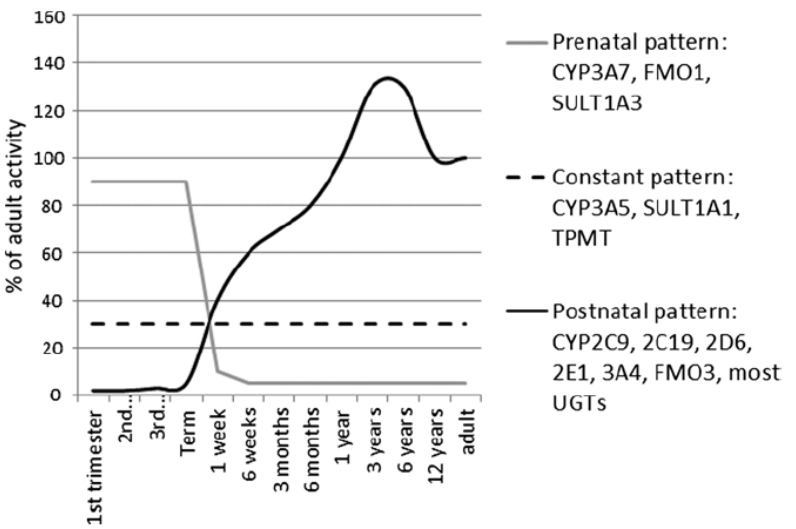
POTENTIAL RESEARCH NEEDS

- Chemical Metabolism Prediction Tools
  - Isozyme-level predictions
    - isozymes relevant for chemical domains of interest
    - Assess against in vitro and/or in vivo data; Assess IVIVE approach
- Ontogeny Data
  - Identify needs (isozymes relevant for environmental pollutants/chemicals)
    - Data generation: resources; quality assessment
    - Sufficient data to discern variability within specific lifestages?
- Genetic Polymorphisms (for chemical domains of interest)
- Looking beyond Plasma C_{ss} → Target Tissue
- Physiology Data
  - Mine available resources; Supplement as necessary
- Exposomic Considerations
  - Cumulative and/or Co-exposures / Health Status / Lifestyle Effects
- Integrative Database and Tool Development



- Bioavailability- fraction of drug that enters the systemic circulation; =(
- Xenobiotic- foreign to the body
- Distribution depends on: lipophilicity, blood flow, capillary permeability, plasma & tissue binding, vol of distribution
  - Vd=(amt drug in body/[plasma drug]); helps determine if mostly in tissue vs plasma
  - High MW drugs tend to be protein bound
- Km=[S] at ½ Vmax; Michaelis constant; https://www.chem.purdue.edu/courses/chm333/Spring%202013/Lectures/Spring%202013%20Lecture%2015.pdf