In vitro-in vivo extrapolation (IVIVE) for neurodevelopment: Toxicokinetics and *in vitro* point of departure evaluation of putative developmental neurotoxicants

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Background

- Brain development spans from the 1st trimester of pregnancy through adolescence, with major windows of susceptibility known to exist from the 2nd trimester through the 1st few years of life.
- Although new approach methodologies (NAMs) are being increasingly employed to evaluate developmental neurotoxicity (DNT), these assays lack two critical barriers that modulate concentrations at the site of brain development—the blood brain barrier (BBB) & fetoplacental barrier.

→ **A specialized *in vitro-in vivo* extrapolation (IVIVE) approach combined with dosimetric modeling is required to estimate target site concentrations of relevance for DNT NAMs**

- This IVIVE model can then be applied to translate *in vitro* points of departure to administered equivalent doses (AEDs) for direct comparison to estimated *in vivo* exposures.

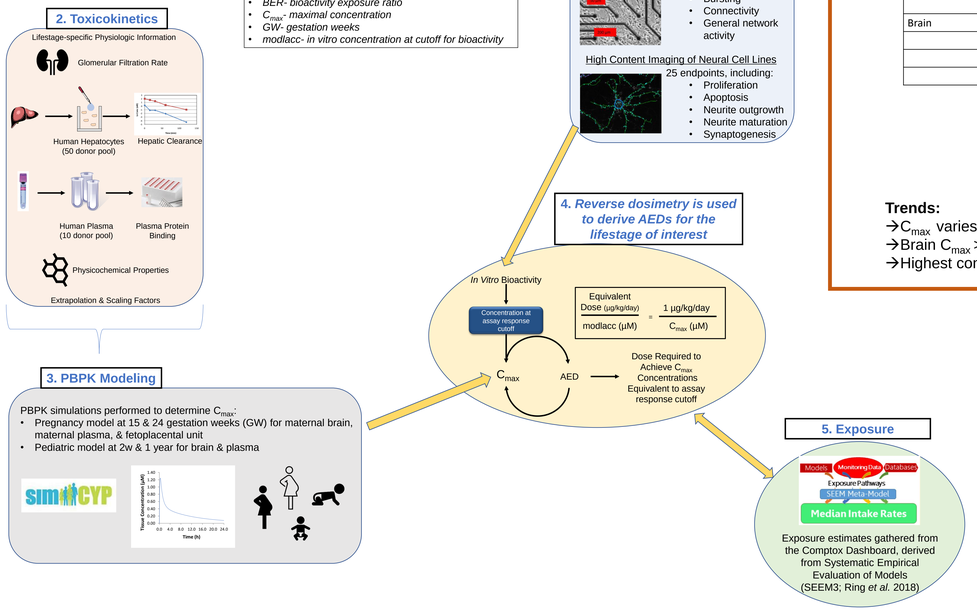
Approach

- In this proof of concept, 81 compounds that elicited *in vitro* DNT NAM activity, from an initial set of ~400 chemicals, and for which *in vitro* toxicokinetic data exist, were incorporated into a physiologically-based pharmacokinetic (PBPK) modeling approach to estimate fetoplacental, brain, and plasma concentrations during various lifestages in fetus, child, or mother. Reverse dosimetry was then employed to derive AEDs, which provide an *in vivo* dose metric for direct comparison to anticipated exposures, providing an ad hoc margin of exposure estimate useful in risk prioritization.

IVIVE Approach

1. Compounds that elicited bioactivity in US EPA DNT NAMs identified
2. Toxicokinetic data gathered from prior efforts (Wetmore *et al*, 2012) & curated
3. PBPK modeling performed using toxicokinetic data to predict C_{max}
4. AED derived using reverse dosimetry based on modlacc from DNT NAMs & C_{max} for lifestages of interest
5. BER determined from comparison between AED and exposure estimates

- *AED- administered equivalent dose*
- *BER- bioactivity exposure ratio*
- *C_{max}- maximal concentration*
- *GW- gestation weeks*
- *modlacc- in vitro concentration at cutoff for bioactivity*



Preliminary Results

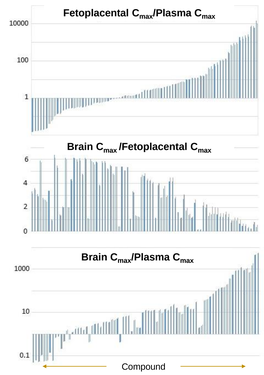
3. Compartment-specific PBPK C_{max} predictions during critical windows of neurodevelopment

- Concentration-time profiles are described by ordinary differential equations that account for blood flows & partition coefficients
- Simcyp PBPK modeling to derive C_{max}
  - Pregnancy model for fetoplacental compartment, maternal brain, & maternal plasma
  - Pediatric model for brain & plasma
- Simcyp pregnancy model
  - Healthy Caucasian pregnant females aged 18-45; 0-40 GW
  - Algorithms describe changes in anatomy, biochemistry, & physiology over course of pregnancy
  - Fetoplacental unit includes fetus, placenta, & amniotic fluid
- Simcyp pediatric population
  - Birth to 25 years
  - Scaled to Northern European Caucasian population
  - Algorithms describe ontogeny & changes in blood flows & tissues

C_{max} Distributions				
Compartment	Age	Median (nM)	Min (nM)	Max (nM)
Plasma	15GW*	1.26	6.15E-4	49.8
	24GW*	1.20	5.33E-4	48.3
	2w	1.46	3.83E-3	51.2
	1y	1.36	3.80E-3	49.7
Fetoplacental	15GW	3.28	6.48E-3	112
	24GW	3.34	6.54E-3	71.6
Brain	15GW*	8.90	6.73E-3	38.0
	24GW*	8.98	6.88E-3	37.3
	2w	3.98	5.13E-3	19.6
	1y	4.97	5.74E-3	18.0

*Maternal

C_{max} Compartmental Distribution Comparison During Pregnancy

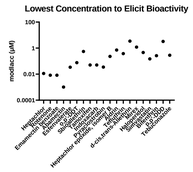


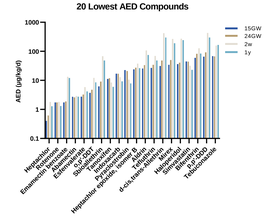
Trends:
→C_{max} varies over 6 orders of magnitude
→Brain C_{max} >Fetoplacental C_{max} >Plasma C_{max}
→Highest concentrations found in fetoplacental compartment

4. Linking toxicokinetics to bioactivity: calculation of AEDs

$$AED = modlacc(\mu M) \times \left(\frac{1\mu g/kg/d}{\mu M\ Cmax}\right)$$

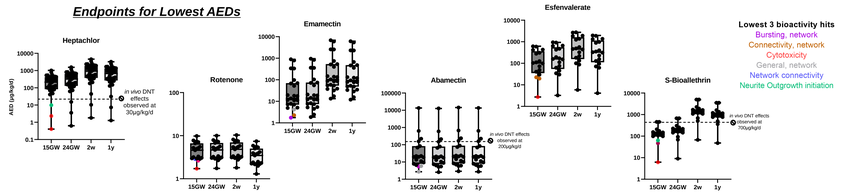
- C_{max} in fetoplacental compartment (15 & 24GW) or brain (2w & 1y olds)





Key findings:
→AEDs ranged from 0.4 µg/kg/d for heptachlor to 2E6 µg/kg/d for triamcinolone
→Lowest AEDs generally found for the earliest lifestage examined
→AED driven largely by bioactivity

Endpoints for Lowest AEDs

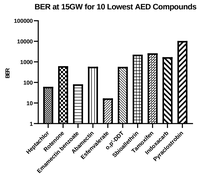


- Plots depict AEDs for all positive endpoints for the 8 lowest AED compounds. The 3 lowest AEDs for each compound are color-coded to indicate their specific endpoint.
- Concentrations that produce *in vivo* effects gathered from ToxRefDb & Mundy *et al,* 2015

Key findings:
→Lowest AEDs generally for endpoints of cytotoxicity, network connectivity, & general network activity
→*In vivo* effects found to be within range of AEDs that elicited bioactivity in DNT assays

5. Relating bioactive concentrations to exposures: estimations of BERs

$$BER = \frac{AED\left(\frac{\frac{mg}{kg}}{d}\right)}{Exposure\ \left(\frac{\frac{mg}{kg}}{d}\right)}$$



- Provides comparison between anticipated external exposures & exposures needed to elicit bioactivity
- Uses AED at 15GW for most potent DNT assay
- Exposure predictions derived from SEEM (Ring, 2018)
- Exposure for 95th %ile for Ages 20-65 & Reproductive Age Females

Key findings:
→One chemical, esfenvalerate, shows a relatively low BER of 17; 2 chemicals—heptachlor & emamectin benzoate—have BERs of ~100; the remaining chemicals have BERs of 500 or more

Conclusions & Future Directions

- We have developed a novel approach to compare concentrations in *in vitro* DNT assays to *in vivo* levels during critical periods of brain development, allowing for determination of *in vivo* exposures that could elicit bioactivity. C_{max} varies widely across this set of DNT-relevant compounds, with chemicals preferentially partitioning into the brain
- *In vivo* DNT effects are within range of AEDs determined
- This approach could be used in risk assessment prioritization of chemicals of concern for DNT

Dosimetric models developed for this purpose need to consider:

- *Fetoplacental transfer & BBB passage*
- *Dynamic nature of developing brain & barriers during critical windows of brain development*
- *Impact of passive & active permeability (i.e. transporter involvement) on chemical bioavailability & target tissue accumulation*
- *Impact of metabolic & transporter ontogenies across relevant lifestages & consequent modulation of target site concentrations*

Future efforts will:

- Incorporate passive & active permeability (e.g., Caco-2 data) to refine fetal brain concentrations
- Incorporate enzyme-specific toxicokinetic data to evaluate variation across early lifestages
- Expand approach to additional chemicals

References

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