Environmental Protection Agency

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

Kreutz, A.[a], Shafer, T.J., Paul-Friedman, K., Wambaugh, J., & B.A. Wetmore [b] [a]Oak Ridge Institute for Science and Education, Oak Ridge, TN; [b] US Environmental Protection Agency, Office of Research & Development, Center for Computational Toxicology & Exposure, RTP, NC

Anna Kreutz | National Institute of Environmental Health Sciences, Division of Intramural Research-Division of the National Toxicology Program | anna.kreutz@nih.gov | 984-287-4124

BACKGROUND: *using new approach methodologies (NAMs) to predict concentrations of chemicals that could elicit developmental neurotoxicity (DNT) in humans requires a novel IVIVE approach*

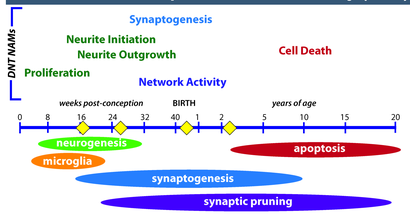
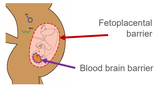


Fig. 1. Timeline of neurodevelopment. Neurodevelopmental events are plotted in lower half. Processes assessed by *in vitro* DNT NAMs are plotted above in corresponding colors. Yellow diamonds indicate ages assessed with DNT-IVIVE approach.

Brain development ranges from the first trimester of pregnancy through adolescence, with major windows of susceptibility spanning from the second trimester through the first few years of life.

Although NAMs are being increasingly employed to evaluate DNT, these assays largely lack the complex physiology of the *in vivo* scenario, which notably includes two dynamic barriers—the fetoplacental barrier and the blood-brain barrier (BBB). *No approach exists to translate in vitro concentrations in DNT NAMs into in vivo doses at the site of brain development during critical windows of susceptibility.*



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

APPROACH

In this proof of concept, 92 compounds that elicited bioactivity in DNT NAMs, for which *in vitro* toxicokinetic data exist, were incorporated into a physiologically-based pharmacokinetic (PBPK) modeling approach to estimate plasma, brain, and fetal tissue concentrations during major windows of susceptibility in fetus, child, and mother. Reverse dosimetry was employed to derive administered equivalent doses (AEDs), which provide estimations of human *in vivo* exposures that could elicit bioactivity at the site of brain development for direct comparison to anticipated exposures through bioactivity to exposure ratios (BERs)—a margin of exposure metric that could be used in setting testing priorities for chemicals of concern for DNT.

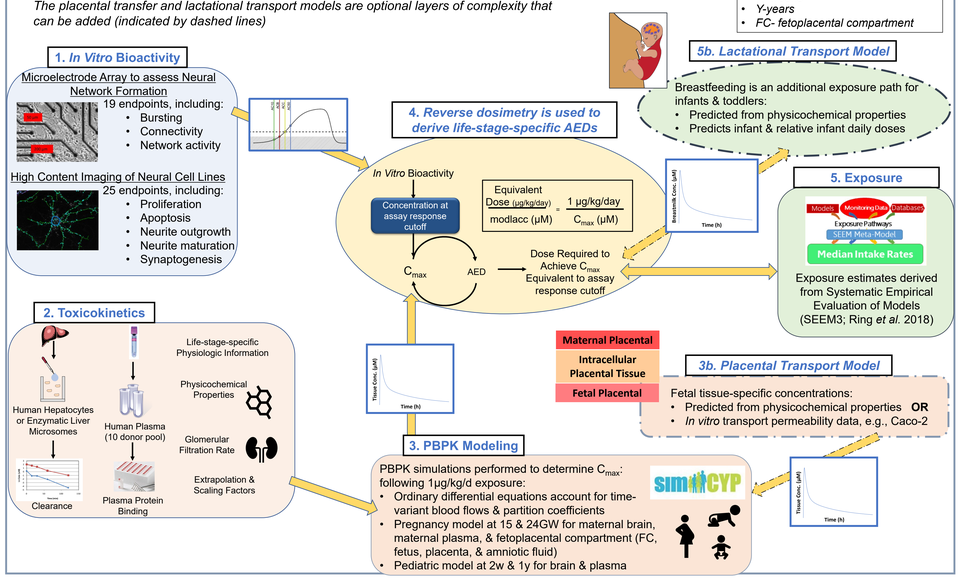
IVIVE APPROACH: *making use of in vitro bioactivity and toxicokinetic data in conjunction with PBPK modeling to derive dosages that could elicit bioactivity in vivo and margin of exposure metrics*

Fig. 2. DNT-IVIVE workflow.

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 response cutoff from DNT NAMs & C_{max} for life-stages of interest
5. BER determined from comparison between AED and exposure estimates

The placental transfer and lactational transport models are optional layers of complexity that can be added (indicated by dashed lines)

- *AED- administered equivalent dose*
- *BER- bioactivity exposure ratio*
- *C_{max}- maximal concentration*
- *GW- gestation weeks*
- *W-weeks*
- *Y-years*
- *FC- fetoplacental compartment*



RESULTS: *prediction of chemical distributions in DNT target tissues which are used to derive in vivo dosages that could elicit DNT bioactivity and margin of exposure metrics*

PBPK Predictions of Chemical C_{max} Distributions at Sites of Brain Development

		Clearance System Input		Fetal Transfer	
		Hepatocytes	Enzymatic Liver Microsomes	Predicted	Caco-2*
Compartment	*Age*	*Med (Min-Max) nM*			
Plasma	15GW	1.57 (0.005-49.9)	0.53 (0.001-40.8)		
	24GW	1.83 (0.005-48.1)	0.53 (0.001-39.7)		
	2w	2.14 (0.006-49.9)	1.09 (0.004-44.8)		
	1y	1.98 (0.006-49.9)	0.74 (0.002-43.9)		
Brain	15GW	9.07 (0.002-34.6)	2.41 (0.005-23.3)	1.36 (0.002-6.98)	1.11 (0.002-6.9)
	24GW	9.56 (0.003-34.0)	2.25 (0.004-24.2)	1.26 (0.001-6.92)	1.23 (0.002-6.96)
	2w	3.67 (0.002-1.68)	2.49 (0.06-8.9)		
	1y	5.60 (0.003-18.9)	2.49 (0.02-48.7)		
Fetoplacental	15GW	2.57 (0.001-78.2)	1.08 (0.001-48.7)		
	24GW	2.66 (0.001-48.4)	1.15 (0.001-34.7)		

Table 1. Distribution of C_{max} values predicted using the different PBPK models Target site concentrations for the different models for gestational (blue) and pediatric (brown) life-stages. *Subset of available chemicals.

Key Findings:

- C_{max} spans 4 orders of magnitude
- Concentrations generally higher at 15GW & 1y
- Highest developmental concentrations in fetoplacental compartment (FC)
- C_{max} higher in brain & FC

Placental Transport model

- Fetal brain concentrations 7th/13 tissues
- Fetal brain concentrations generally < FC
  - Fetal brain levels tighter than FC
  - Fetal brain > FC for 31 compounds
- Caco-2 & Literature-derived fetal brain concentrations similar to predicted

Compartment	15GW	24GW
	Med (Min-Max) nM	
Pancreas	4.01 (0.002-13.7)	2.95 (0.002-11.3)
Adipose	3.93 (0.002-19.4)	2.83 (0.002-17.1)
Skin	2.74 (0.003-7.80)	2.18 (0.002-9.40)
Liver	2.12 (0.002-7.99)	1.78 (0.002-8.16)
Gut	2.15 (0.002-7.07)	1.69 (0.002-7.07)
Brain	1.35 (0.002-6.98)	1.69 (0.001-6.92)

Table 2. C_{max} values in top 5 fetal tissues, as well as fetal brain, using the predicted fetal transfer approach.

Lactational model

- Infant Daily Doses span 8 orders of magnitude
  - 2 µg/kg/d (6E-7-11.4)

- Drivers of distribution
  - Chemical test set has higher than average lipophilicity (log $P_{o:w}$)
  - Chemical lipophilicity (higher log $P_{o:w}$) shows a positive correlation with brain & FC concentrations
  - Neutral chemicals more readily partition into the brain & FC than do acids and bases
  - Brain concentrations correlate with fraction unbound

Linking Toxicokinetics to Bioactivity: Calculation of Administered Equivalent Doses (AEDs)

- **Estimate of *in vivo* dosages that could elicit bioactivity in humans**

Life-stage specific AEDs for Variations of DNT-IVIVE Approach

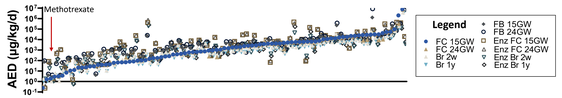


Fig. 3. Distribution of AEDs for the lowest bioactive endpoints across the test using the different DNT-IVIVE approaches. AEDs are based on concentrations in the FC or fetal brain (FB) at 15 and 24GW, and in the brain (Br) at 2w and 1y using hepatic or enzymatic (Enz) clearance rates derived from *in vitro* toxicokinetic assays and *in silico* predictions. AEDs are plotted from lowest at 15GW on the left, to highest on the right.

Key Findings:

- AEDs ranged from 0.25 µg/kg/d for heptachlor to 8E6 µg/kg/d for triamcinolone
- Regression analysis shows AED to be driven by bioactivity to a greater extent than toxicokinetics

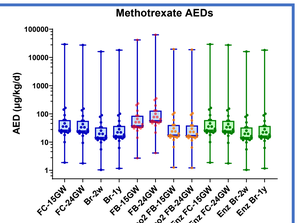


Fig. 4. Box and whisker plots show AEDs for all bioactive endpoints from DNT NAMs using the different DNT-IVIVE approaches for Methotrexate, which has the 3rd lowest 15GW AED. AEDs are based on concentrations in the FC (blue points) or FB (red points) at 15 and 24GW, & in the brain (Br) at 2w & 1y of age using hepatic (blue border) or enzymatic (green border) clearance. FB concentrations can additionally be predicted from Caco-2 passive permeability (orange).

Relating Bioactive Concentrations to Exposures: Estimations of Bioactivity Exposure Ratios (BERs)

- Provides comparison between anticipated external exposures & exposures needed to elicit bioactivity
- Uses AED at most sensitive life-stage for most potent DNT assay
- Exposure predictions derived from SEEM-3 (Ring, 2018) for Reproductive Age Females
  - Breastmilk exposure from lactational transport model incorporated for 2w & 1y life-stages
- **Can be used as a margin of exposure metric in setting testing priorities for chemicals of concern for DNT**

Key Findings:

- Esfenvalerate & heptachlor show relatively low BERs of 18 & 35 for gestational ages
- Methotrexate has a BER of 99 for pediatric life-stages
- Remaining chemicals have BERs of > 100, with most >1000

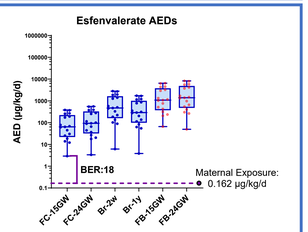


Fig. 5. Box and whisker plots show distribution of AEDs in relation to exposure estimates for Esfenvalerate. BER based on lowest AED.

DNT-IVIVE: *in vitro-in vivo* Comparisons to Evaluate Predictivity

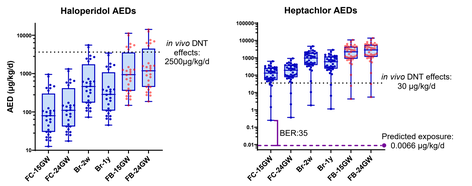


Fig. 6. Box and whisker plots show distribution of AEDs for haloperidol and heptachlor in relation to doses shown to elicit DNT effects *in vivo*. Exposure estimates are additionally provided for heptachlor, which has a relatively low BER. *Predicted exposure is not plotted for haloperidol due to scale.*

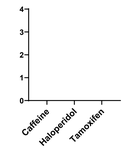


Fig. 7. Fold difference between breastmilk levels obtained via PBPK modeling using the lactational model in comparison to literature-reported *in vivo* concentrations in humans.

Key Findings:

- ***In vivo* effects found to be within range of AEDs that elicited bioactivity in DNT assays**
  - DNT-NAM points of departure (PODs) are more conservative than *in vivo*-derived PODs
- **Breastmilk levels fall within 3X of those predicted by DNT-IVIVE approach**

Conclusions & Future Directions

- This DNT-IVIVE approach can be used to translate *in vitro DNT points of departure to in vivo human equivalent* dosages for NAM and margin of exposure evaluations
- The concordance between our model-derived AEDs and lactational exposure outputs and *in vivo*-derived DNT PODs and breastmilk concentrations, respectively, demonstrates this approach holds potential for setting testing priorities for chemicals of concern for DNT
- This predictive toxicology approach is versatile:
  - *Incorporates intricacies of brain development, allowing for life-stage, chemical, and endpoint-specific estimations of in vivo exposures that could elicit bioactivity at the site of brain development*
  - *Can be integrated with previously published or future generated bioactivity and toxicokinetic data*
  - *Allows for varying degrees of complexity based on risk evaluation needs and availability of in vitro data*
- The data gathered here could be used to build models to predict brain distribution of environmental chemicals

This dosimetric model considers:

- *Fetoplacental, BBB, & lactational transfer*
- *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 life-stages & consequent modulation of target site concentrations*

Future efforts will:

- Incorporate further passive & active permeability data (e.g., Caco-2) to refine fetal brain concentrations
- Use the data gathered here to build predictive biological models of brain distribution of environmental chemicals

Acknowledgements & Funding

Dr. Kreutz would like to thank her current supervisors, Drs. Guang Hu[1] and Erik Tokar[2] for their continued support of this work.

Funding for this work was provided by ORISE and the Environmental Protection Agency's Chemical Safety for Sustainability Research Program

[1]NIEHS, Division of Intramural Research

[2]NIEHS, Division of National Toxicology Program

References

(1) Wetmore BA et al. 2012 Toxicol Sci. Jan;125(1):157-74. PMID: 21948869.
(2) Frank et al. 2017 Toxicol Sci. Nov 1;160(1):121-135. PMID: 28973552.
(3) Harrill et al. 2018 Toxicol Appl Pharmacol. Sep 1;354:24-39. PMID: 29626487.
(4) Mundy WR et al. 2015. Neurotoxicol Teratol. Nov-Dec;52(Pt A):25-35. PMID: 26476195.
(5) Ring CL et al. 2019 Environ Sci Technol. 2019 Jan 15;53(2):719-732. PMID: 30516957.
(6) Abduljalil K et al. Clin Pharmacokinet. 2019 Feb;58(2):235-262. PMID: 29987449.