National Institute of Environmental Health Sciences
Division of Translational Toxicology

In Vitro to In Vivo Extrapolation for Developmental Neurotoxicity: A Comparison of Physiologically Based Pharmacokinetic Models

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Background and Purpose

A battery of in vitro assays has been developed for assessing developmental neurotoxicity (DNT), with the aim of replacing traditional in vivo guideline studies for DNT risk assessment. These in vitro assays hold many advantages over costly and lengthy in vivo studies. However, at present, there is no standardized approach to translate in vitro bioactive concentrations into in vivo dosages.

- In prior work we developed an in vitro to in vivo extrapolation (IVIVE) approach for DNT using the Simcyp™ physiologically-based pharmacokinetic (PBPK) modeling platform.
- Here we sought to transfer this DNT-IVIVE approach to other PBPK modeling programs, GastroPlus® (GP) and the U.S. Environmental Protection Agency's (EPA's) open-source high-throughput toxicokinetics (httk) package, to:
  - Evaluate model differences and limitations.
  - Enhance adherence to FAIR (findable, accessible, interoperable, reusable) principles.
  - Explore the degree of population variability in this DNT-IVIVE approach.
  - Calculate metrics for risk assessment.

Methods Overview

Methods

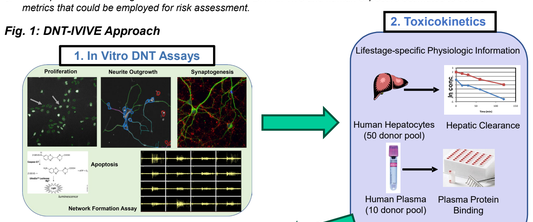
A set of chemicals was identified based on:

1. *Identification of DNT bioactivity from ToxCast data on EPA in vitro DNT assays.*
2. *Existence of in vitro toxicokinetic data generated by EPA.*

Chemicals were processed through our DNT-IVIVE approach (Fig. 1), incorporating:

3. *PBPK modeling at four life-stages spanning critical periods of neurodevelopment in GP and httk to derive toxicokinetic metrics at the site of brain development.*
4. *IVIVE to calculate administered equivalent dosages (AEDs).*
5. *A comparison of AEDs against doses shown to elicit DNT in vivo and human exposure estimates to derive metrics that could be employed for risk assessment.*

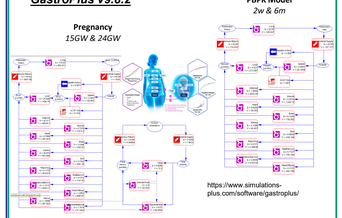
Fig. 1: DNT-IVIVE Approach



Approach

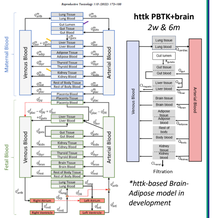
- Experimental toxicokinetic data for hepatic clearance and fraction unbound in plasma (f_{up}) incorporated
  - Model defaults used for remaining parameters (e.g. physicochemical properties, body weights)
- 1 mg/kg/day oral dose modeled for 24 hr
- 15 and 24 gestation weeks (GW) to model 2nd and 3rd trimester, respectively, using pregnancy PBPK models
  - In httk, fetal tissue chemical distributions were consolidated into a lumped fetal compartment
  - Metrics: plasma, fetal venous, fetus, brain (httk only)
- 2w and 6m postpartum modeling in standard PBPK models scaled by age (GP) or body weight (httk)
  - Preliminary httk-brain-adipose model used to derive brain concentrations in httk
  - Metrics: plasma, brain

GastroPlus v9.8.2



- Commercial tool from Simulations Plus
- Chemical-specific predictions from ADMET Predictor (Simulations Plus) read into GP
- Pediatric ontogeny (e.g. physiology, metabolism) considered in standard PBPK model based on age

httk v2.2.2



- Open-source R package
- Predictions from OPERA & curated data
- Pregnancy model provides fetal-tissue specific concentrations
- Assume fraction absorbed of 1

PBPK Modeling Results

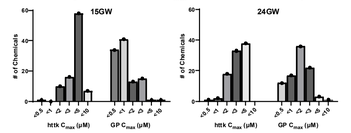
Table 1. C_{max} Distribution by PBPK model, Compartment, and Life-stage

	Med (Min-Max) C_{max} (uM)							
	Pregnancy				Pediatric			
Compartment	GP 15GW	httk 15GW	GP 24GW	httk 24GW	GP 2w	httk 2w	GP 6m	httk 6m
Plasma	1.78 (0.21-40.72)	1.89 (0.19-19.96)	1.52 (0.11-38.91)	1.74 (0.17-18.42)	1.32 (0.01-37.89)	2.76 (0.22-37.22)	1.69 (0.02-41.44)	3.03 (0.22-38.66)
R^2, RMSE	0.77, 1.30		0.74, 1.28		0.40, 4.62		0.47, 4.00	
Fetal Venous	1.40 (0.20-11.83)	1.20 (0.27-12.45)	1.72 (0.01-16.63)	0.96 (0.27-18.42)				
R^2, RMSE	0.60, 1.09		0.71, 0.84					
Fetus	0.64 (0.10-6.18)	3.73 (0.32-6.69)	1.39 (0.10-6.01)	2.78 (0.34-4.96)				
R^2, RMSE	0.07, 1.15		0.39, 0.69					
Brain		2.22 (0.28-5.34)		1.76 (0.31-4.68)	2.29 (0.01-9.67)	3.54 (1.49-9.04)	3.28 (0.02-12.93)	3.58 (1.23-9.20)
R^2, RMSE					0.09, 1.54		0.13, 1.59	

*R^2 and RMSE values for the correlation between httk and GP predicted C_{max}.

- Predicted maternal plasma and fetal venous C_{max} values at 24GW fell within 3.2-fold, or "on the order," (Wambaugh et al., 2015) of one another for 90/91 chemicals, highlighting the similarity of predictions between the two programs.
  - Fetal concentrations are similar in the two pregnancy models at 24GW but show greater variation at 15GW.
  - Predictions of plasma and brain C_{max} at 2w and 6m postpartum are less well-aligned between GP and httk than in the pregnancy model based on median and R^2 values.
- The distribution of C_{max} values in the fetal compartment is broader in GP than httk, with more C_{max} values <1 in GP than httk (Fig. 2).

Fig. 2: Distribution of C_{max} Values in the Fetal Compartment



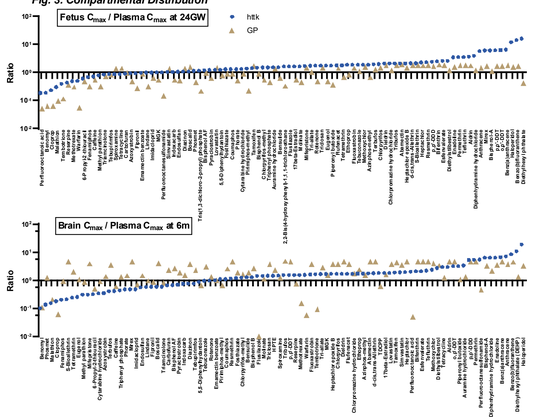
Compartmental Partitioning

- Chemicals preferentially partition into the fetal compartment as compared to plasma in httk (>1), whereas in GP, chemical concentration is similar in plasma and fetus (Table 2). This may in part be attributed to the distribution of fetal C_{max} values (Fig. 2).
- Chemicals preferentially partition into the brain compartment in both GP and httk.

Table 2. Chemical Compartmental Partitioning

	24GW C_{max} Fetus/Pl		6m C_{max} Brain/Pl	
	httk	GP	httk	GP
Avg	2.22	0.98	2.00	2.38
Med	1.57	0.91	1.42	2.17
Min	0.18	0.05	0.10	0.05
Max	16.04	1.86	18.79	4.71

Fig. 3: Compartmental Distribution



Assessment of Model Parameters to Identify Underlying Differences Between GP and httk

Blood flows, fraction bioavailable (F_{bio}), physicochemical properties (e.g. logD), volumes, and calculation of adjustment for f_{up} and fraction unbound in the incubation ($f_{unbound}$), renal Cl_{int}, and tissue partition coefficients (K_p) were evaluated.

- Methods for calculation of K_p differ between the two programs but provide similar values (liver unbound K_p (K_{pu}) R^2 = 0.70; brain K_{pu} R^2 = 0.54) (Pearce et al, 2017).
- F_{up} adjustment, used in calculation of K_p's, is similar for the two programs (R^2 = 0.98).
- Programs perform similarly to defaults when parameterized with physicochemical properties.
- F_{bio} was identified as a parameter that might explain major outliers as the 3 chemicals with the greatest differences were those with the predicted F_{bio}, whereas httk assumes a F_{bio} of 1 (Fig. 4, purple oval).
  - Most of the F_{bio} are >90%, so the httk assumption is similar.

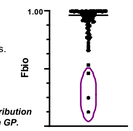


Fig. 4: Distribution of F_{bio} in GP.

Consideration of Population Variability

Interindividual variability in toxicokinetics is known to impact chemical toxicity, raising the question of default uncertainty factors and the degree of risk within a population. To address the extent of population variability and better understand chemical risk, population variability was modeled in GP.

- Five physicochemically and toxicokinetically diverse compounds were selected (Table 3).
- Population simulations were performed using Monte Carlo simulation in GP using default ranges for a population of 100 at each of the ages.
- The population variability ranges from 40% to 70% from the 50th to 95th percentile for these chemicals in the four compartments of interest (Table 3).
- No significant differences in the degree of variability are seen between the different compartments or the four ages for this subset of chemicals.

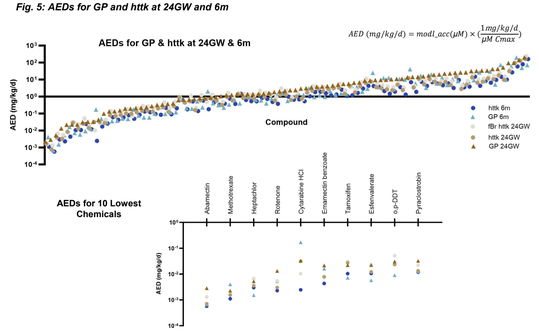
Table 3. Distribution of C_{max} Values Across a Population in GP at 24GW.

	A) Chlorpyrifos	B) Fipronil	C) MGK 264	D) Tetracycline	E) Pyraclostrobin
	Neutral, low Cl_{int} & f_{up}, 4.7 logP	Neutral, Cl_{int}=0, low f_{up}, 4 logP	Neutral, high Cl_{int}, low f_{up}, 3.7 logP	MPA, Cl_{int}=0, f_{up}=0.5, logP=-1.3	MPB, high Cl_{int}, low f_{up}, logP=4
	C_{max} uM 50th-95th %ile; % variation				
Maternal Plasma	0.44-0.61; 37%	0.70-0.91; 29%	0.38-0.53; 39%	0.26-0.44; 67%	0.63-0.88; 42%
Maternal Brain	3.85-5.28; 37%	1.34-1.84; 38%	1.35-1.93; 37%	0.30-0.50; 70%	2.51-3.46; 44%
Fetus	0.67-0.92; 38%	0.25-0.37; 48%	0.20-0.28; 56%	0.37-0.63; 72%	0.37-0.58; 37%
Fetal Venous	0.35-0.51; 43%	0.33-0.46; 40%	0.27-0.37; 41%	0.26-0.44; 68%	0.43-0.61; 39%

Hepatic clearance (Cl_{int}); fraction unbound in plasma (f_{up}); monoprotic acid (MPA); monoprotic base (MPB)

IVIVE: Administered Equivalent Dosages (AEDs) Provide Estimates of Human Exposures that Could Elicit DNT

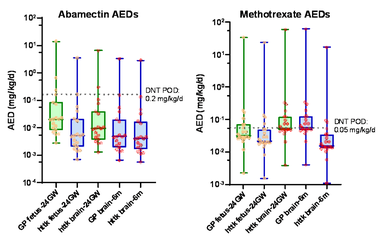
Fig. 5: AEDs for GP and httk at 24GW and 6m



- While AEDs vary by chemical, life-stage, and model, httk generally provides lower (and thus more conservative) predictions of AED than GP.
- AEDs are generally lower for infant brain at 6m than for fetus at 24GW.

In Vivo PODs Align with In Vitro AEDs

Fig. 6: Comparison of DNT In Vitro-Derived AEDs Against In Vivo PODs



Range of AEDs derived from DNT-IVIVE of all endpoints active in the in vitro DNT assays for the two chemicals with the lowest AEDs, abamectin and methotrexate, for both GP (green) and httk (blue) at 24GW and 6m for the DNT-specific compartments—fetus (tan) and brain (red).

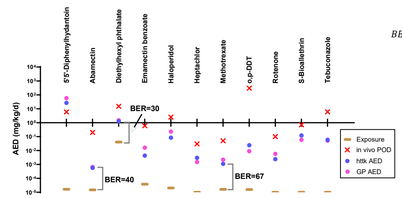
- In vivo DNT PODs fall within the range of AEDs for bioactive endpoints for both programs, showing the concordance of in vitro-derived DNT-IVIVE predictions with in vivo data.
- This predictive toxicology DNT-IVIVE approach incorporates the intricacies of fetal development and allows for life-stage, chemical, and endpoint-specific estimations of in vivo exposures that could elicit bioactivity at the site of brain development.

Incorporation of Exposure Information for Risk Assessment Considerations

- Due to the lack of in vivo data for DNT, in vitro-derived AEDs from the most sensitive in vitro endpoint were compared against points of departure (PODs) from in vivo DNT studies as an assessment of model predictivity.
- PODs exceeded in vitro-derived AEDs, suggesting in vitro AEDs may be more conservative for DNT risk assessment.
- Bioactivity exposure ratios (BERs) provide quantitative metrics for risk assessment. Based on SEEM2 exposure predictions (Ring et al., 2019), the majority of chemicals had BERs greater than 100, while three chemicals had BERs less than 100 and thus may be of relatively higher concern.

Fig. 7: Comparison of IVIVE-derived AEDs to In Vivo Effective Concentrations and Exposures

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



Results and Discussion

- The httk and GP PBPK pregnancy models performed similarly at 24GW and for plasma concentrations.
  - Fetal concentrations showed a greater difference than plasma, which may be attributed to the separation of the fetus into individual tissue compartments in httk, whereas GP provides a single "fetal tissue" estimate.
  - Poorer alignment was seen for 2w and 6m fetal estimates. This may be due to the lack of consideration of metabolic and physiologic ontogeny in the general httk PBPK model.
- Concordance between models provides greater confidence in model predictions; lack of concordance suggests a need for further assessment of predictions.
- Two notable differences were observed between the models:
  - GP provides lower predictions of fetal C_{max} than httk.
  - The utility of httk is limited for the neonatal life-stage as the general PBPK model does not consider ontogeny.
- Minimum AEDs generally fell below in vivo DNT PODs, suggesting that using in vitro metrics may be more conservative than in vivo data for risk evaluation.

Conclusions

PBPK modeling was performed in GP and httk to assess the broader applicability of our DNT-IVIVE approach and evaluate model differences and limitations.

- Chemical C_{max} values in fetus and brain predicted by both models, particularly in httk, frequently exceeded plasma concentrations, suggesting plasma may not be an appropriate metric for estimating DNT risk.
- httk generally produced the lowest AEDs, thereby providing a more conservative approach than GP for DNT, as might be preferred for risk assessment.
  - However, AEDs are similar across platforms, with in vivo PODs falling in the range of in vitro-derived AEDs for both programs, suggesting this DNT-IVIVE approach is readily transferable across modeling platforms, albeit with varying limitations regarding model accessibility and complexity, which must be considered appropriately within the context of use.
  - A multi-model approach, as we have performed here, can build confidence in predictions and point to critical factors that determine tissue concentrations.
- Transparency around model assumptions and limitations is critical for acceptance of these models by decision-makers due to the complexity and lack of available data to validate these models. Open-source tools are therefore critical for such applications.

Future Directions:

- This DNT-IVIVE approach can be integrated with future-generated bioactivity and toxicokinetic data and allows for varying degrees of complexity based on chemical risk evaluation and availability of in vitro data.
- Experimental data on chemical distribution, particularly in humans and for environmental chemicals, is needed to provide greater confidence in these models.

References and Acknowledgments

Kapraun DF et al. 2022. Reprod Toxicol. 113:172-188. https://doi.org/10.1016/j.reprotox.2022.09.004.

Pearce RG et al. 2017. J Pharmacokinet Pharmacodynam 44(6):549-565. https://doi.org/10.1007/s10928-017-9548-7.

Ring CL et al. 2015. Environ Sci Technol 53(2):719-732. https://doi.org/10.1021/acs.est.8b04056.

Wambaugh JF et al. 2015. Toxicol Sci. 147(1):55-67. https://doi.org/10.1093/toxsci/kfv118.

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