In Vitro to *In Vivo* Extrapolation for Developmental Toxicity Potency of Valproic Acid Analogues

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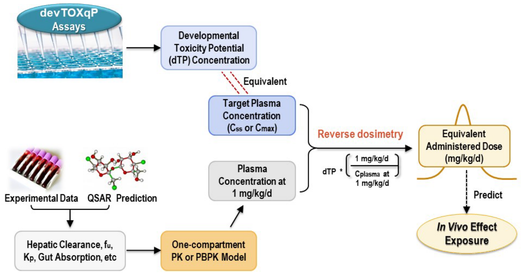
Introduction

- European Union ToxRisk (https://www.eu-toxrisk.eu/) is a European Union (EU)-wide research program driving mechanism-based toxicity testing and risk assessment for the 21st century. EU ToxRisk has developed several case studies to address alternative models in regulatory decision making. One case study is to investigate the teratogenic potency of valproic acid (VPA) analogues that have been tested with the devTOX *quick*Predict™ assay (devTOX^{qP}), a human induced pluripotent stem cell (iPSC)-based assay.
- Previous work showed that the potency ranking from devTOX^{qP} assay was consistent with *in vivo* developmental toxicity potency, but whether the assay could quantitatively predict *in vivo* exposure exerting developmental toxicity was unknown.
- In this study, *in vitro* to *in vivo* extrapolation (IVIVE) was performed to predict the *in vivo* developmental toxicity dose levels by estimating equivalent administered doses (EADs) that would result in maternal and/or fetal blood concentrations equivalent to the developmental toxicity potential (dTP) concentrations derived from the devTOX^{qP} assay **(Figure 1).** The impact of pharmacokinetics and different modeling approaches on EAD prediction was also evaluated.

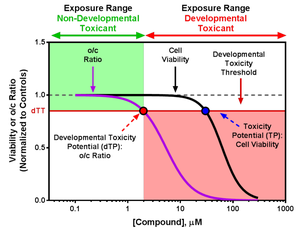
Figure 1. Predicting *In Vivo* EAD Using *In Vitro* Activity Concentration



Data and Pharmacokinetics Model Inputs

In vitro assay data

- The devTOX^{qP} assay is a biomarker-based human pluripotent stem cell assay for developmental toxicity screening (Stemina Biomarker Discovery, Inc.) (Palmer et al. 2013, 2017).
  - The assay measures changes in ornithine and cystine following exposure, represented as ornithine to cystine (o/c) ratio.
  - The o/c ratio is associated to developmental toxicity and used for deriving the development toxicity potential (dTP) concentration.
  - Cell viability is used for deriving the toxicity potential (TP) concentration.



Pharmacokinetics (PK) parameters

- PK parameters from literature data or OPERA model predictions (Mansouri et al. 2018):
  - fu: fraction of chemical unbound to plasma protein.
  - Hepatic clearance and renal clearance.
- Additional PK or physiologically based pharmacokinetics/toxicokinetics (PBPK/PBTK) model parameters, provided by the U.S. Environmental Protection Agency's httk (high-throughput toxicokinetics) R package or commercial software (Pearce et al. 2017; Simulations Plus, Inc.).
  - Uptake rate of chemical from the gut.
  - Tissue:plasma partition coefficients of various tissues (e.g. liver, gut, kidney, etc.).
- *In vivo* data: lowest effective levels (LELs) from *in vivo* developmental toxicity studies (**Table 2**).

Table 1. Input PK Parameter and *In Vitro* Assay Data

CASRN	Chemical Name	dTP (µM)	TP (µM)	dTP / dTP$_{VPA}$	fu[a]	CLinVitro[a] (µl/min/10^6 cells)
1185-39-3	2,2-Dimethylpentanoic acid	784	1745	3.3	0.488	0.00881
142-62-1	Hexanoic acid	838	1022	3.6	0.401	0.00336
149-57-5	2-Ethylhexanoic acid	399	390	1.7	0.245	0.00112
1575-72-0	2-Propyl-4-pentenoic acid	611	636	2.6	0.320	0.00087
31080-39-4	2-Propylheptanoic acid	546	425	2.3	0.210	0.00036
4536-23-6	2-Methylhexanoic acid	976	1631	4.1	0.379	0.00610
591-80-0	4-Pentenoic acid	913	719	3.9	0.640	0.00194[c]
88-09-5	2-Ethylbutyric acid	1071	NA	4.5	0.540	0.00194[c]
97-61-0	2-Methylpentanoic acid	1248	NA	5.3	0.556	0.00662
99-66-1	Valproic acid (VPA)	236	318	1.0	0.243[b]	1.76235E-06[b]

CLinVitro, *in vitro* intrinsic clearance of hepatocytes. [a] Predictions from OPERA QSAR model (Mansouri et al. 2018) unless indicated otherwise; [b] Experimental values from literature (Wetmore et al. 2012); [c]Chemicals are outside of QSAR model applicability domain; values obtained using a median imputation method.

PK Models Used in IVIVE

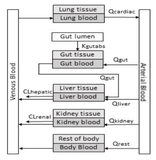
- **Figure 2** shows the structures of the various PK models used in the IVIVE analysis.
- **Figure 2A** shows the open-source one-compartment, population-based PK (PPK) model:
  - Estimates the upper 95th percentile steady-state plasma concentration (Css) following a given dose for a Monte Carlo simulated population that accounts for interindividual physical variability (Wetmore et al. 2012).
  - EADs were calculated that would lead to the total or unbound fraction of Css equal to the dTP concentration from the devTOX^{qP} assay.
    - EAD corresponding to total chemical concentration: $EAD = In\ Vitro\ Effective\ Conc \times \frac{1}{C_{ss}} (mg/kg/day)$
    - EAD corresponding to unbound chemical concentration: $EAD_{f_u} = EAD \times \frac{1}{f_u} (mg/kg/day)$
- **Figures 2B** and **2C** show the open-source standard and pregnancy-specific PBTK models, respectively. Both models are provided by the httk R package. The standard PBTK model is available for both human and rat, but the pregnancy-specific PBTK model is only available for human (Pearce et al. 2017; Kapraun et al. 2019).
  - A standard PBTK model is used for simulating the 1st trimester, and a pregnancy-specific PBTK model is used for simulating the 2nd and 3rd trimesters.
  - Both models were used to calculate EADs that result in a maximum plasma concentration (Cmax) corresponding to the *in vitro* dTPs.
- **Figure 2D** shows the commercial pregnancy PBPK model:
  - A human 10-week gestation model built using GastroPlus™ software (Simulations Plus, Inc.) simulating oral route of exposure in tablet form assuming delayed release.

Figure 2. Structures of Models Used in IVIVE Tool

A PPK model

$$C_{ss} = \frac{\text{Standard oral dose rate (1 mg/kg/day)}}{(GFR * f_u) + \left(Q_{liver} * f_u * \frac{CL_{int}}{Q_{liver} + f_u * CL_{int}}\right)}$$

B HTTK PBTK model



C HTTK pregnancy-specific PBTK model (HTTK.fPBTK)

BW, body weight; CL, clearance; CLint, intrinsic clearance; GFR, glomerular filtration rate; I.V., intravenous injection; Q, blood flow rate; ACAT, advanced compartmental absorption and transit model; V, volume

D GastroPlus™ Pregnancy PBPK model

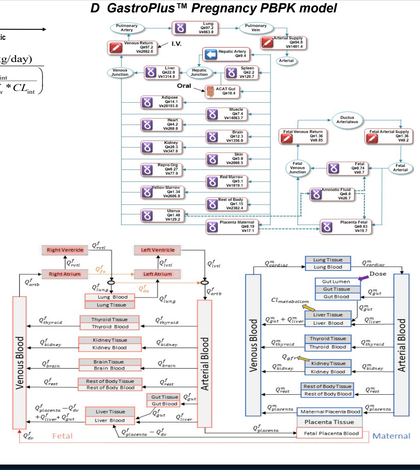
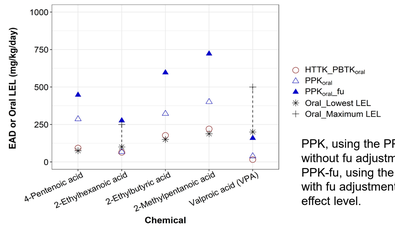


Table 2. EADs Predicted Using Various PK Models, Rat LELs and Human Exposure

Chemical Name	Rat EAD (mg/kg/d): Non-pregnancy model			Rat LEL[a] (mg/kg/d) (oral, repeat, fetal toxicity)	Human EAD (mg/kg/d): Non-pregnancy model			Human EAD (mg/kg/d): Pregnancy model**				Clinical dose (mg/kg/d)
	PPK*	PPK(fu)*	HTTK.PBTK#		PPK*	PPK(fu)*	HTTK.PBTK#	HTTK.fPBTK; maternal Cmax	HTTK.fPBTK; fetal Cmax	GastroPlus™; maternal Cmax	GastroPlus™; fetal Cmax	
2,2-Dimethylpentanoic acid	253	518	206	NA	73	149	96	105	110	28	48	NA
Hexanoic acid	185	461	124	NA	54	134	69	75	76	25	51	NA
2-Ethylhexanoic acid	**68**	**276**	**65**	**100[b] – 250[c]**	19	76	29	31	32	13	36	NA
2-Propyl-4-pentenoic acid	125	390	92	NA	37	116	51	55	55	20	45	NA
2-Propylheptanoic acid	93	442	126	NA	27	129	56	61	63	31	89	NA
2-Methylhexanoic acid	229	606	194	NA	66	176	93	101	105	33	68	NA
4-Pentenoic acid	**286**	447	**93**	**75[d]**	77	121	69	73	74	31	45	NA
2-Ethylbutyric acid	**322**	**596**	**177**	**150[d]**	98	181	109	119	121	37	60	NA
2-Methylpentanoic acid	**401**	**722**	**220**	**188[d]**	117	210	133	146	149	45	69	NA
Valproic acid (VPA)	39	**159**	16	**200[e] – 500[d]**	12	51	11	12	14	7	20	10 - 60

The EAD values are highlighted **in bold blue** when they are within 4-fold of the lowest or highest rat LELs (**bolded**); EAD, equivalent administered dose corresponding to the dTP; LEL, the lowest effect levels that cause adverse effects in fetal development. *The model estimates Css; #The model estimates Cmax; **The pregnancy model simulates a 30-year-old American female with body weight of 63 kg at 10 weeks of gestation. [a]Data were extracted from rat studies with oral, repeat dosing unless indicated otherwise; [b]Data from Pennanen et al. 1992; [c]Data from Hendrickx et al. 1993; [d]Data from Narotsky et al. 1994; [e]Data from Binkerd et al. 1988.

Figure 3. Comparison of Rat EADs to Oral LELs for Selected VPA Analogues



PPK, using the PPK model without fu adjustment.
PPK-fu, using the PPK model with fu adjustment; LEL, lowest effect level.

Results

- The dTP concentration from the devTOX^{qP} assay is very close to or lower than the TP concentration for the majority of VPA analogues **(Table 1).** Therefore, using dTP concentration as the *in vitro* activity concentration in IVIVE analysis provides a more conservative estimate to the rat developmental toxicity LELs than using TP concentration.
- All three rat PK models (i.e., PPK, PPK(fu), HTTK.PBTK) produced rat EADs within four-fold of the LEL range for three of the five VPA analogues. For all five VPA analogues with available LELs, at least one rat PK model produced an EAD within 1.5-fold of the LEL range (**Table 2**).
- The EAD estimate using the rat PPK model with fu adjustment provided the most accurate prediction for rat LEL for valproic acid and 2-ethylhexanoic acid (highest LEL), while the rat HTTK.PBTK model provided the most accurate predictions for rat LELs for the remaining VPA analogues and the lowest LEL for 2-ethylhexanoic acid (**Figure 3**).
- Among all human PK models evaluated, the EAD estimate using the GastroPlus™ pregnancy model simulating maternal Cmax provided the most conservative estimate for human exposure. It also produced an EAD only 1.5-fold less than the lowest clinical dose for VPA.

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Discussion and Conclusion

- IVIVE is a useful tool to evaluate the correlation between *in vitro* and *in vivo* activity for toxicologically relevant endpoints. For chemicals lacking *in vivo* data, IVIVE can be used to predict relevant *in vivo* doses with potential toxicity based on *in vitro assay* measurements, expediting the safety assessment process.
- The close agreement between EAD estimates and rat developmental toxicity LELs for all the VPA analogues with known rat LELs suggests that the dTP of devTOX^{qP} assay in combination with IVIVE approaches could quantitatively predict *in vivo* developmental toxicity potential of VPA analogues.
- The variations among different types of PK/PBPK models for IVIVE are within expected ranges. IVIVE using the open-source HTTK.PBTK model provided the most accurate overall predictions for the rat developmental toxicity LELs of VPA analogues.
- This study highlights the importance of pharmacokinetic considerations in assessing a chemical's developmental toxicity potency based on *in vitro* assays.

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