Development and validation of Mother-foetus PBK model: A case study of PBK read-across for Valproic acid and 2-Ethyl Hexanoic Acid

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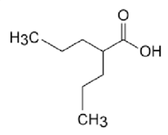
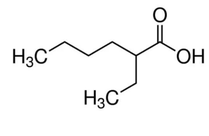
P08-18; Abstract#749

1. Background and Aim:

Physiologically based kinetic (PBK) modelling is a pivotal tool in risk assessments based on New Approach Methodologies to assure safety without the use of animal testing (Middleton et al., 2022).
Read across is one way to improve confidence in PBK model output in the absence of substance-specific data (Paini et al., 2021). Valproic acid (VPA) and 2-Ethyl Hexanoic Acid (2-EHA) both have a similar chemical structure and similar absorption, distribution, metabolism and excretion (ADME) properties (Wu et al., 2022).



Valproic acid



2-Ethyl Hexanoic acid

VPA is an established human teratogen with a plethora of human clinical pharmacokinetic (PK) studies including sparse pregnancy PK studies.

No human PK studies are available for 2-EHA. To fill this data gap the aim of this work was to develop, parameterise and validate a mother-foetus PBK model for 2-EHA based on the structurally similar PK analogue VPA (a PBK read-across). The overall approach is shown in flowchart below.

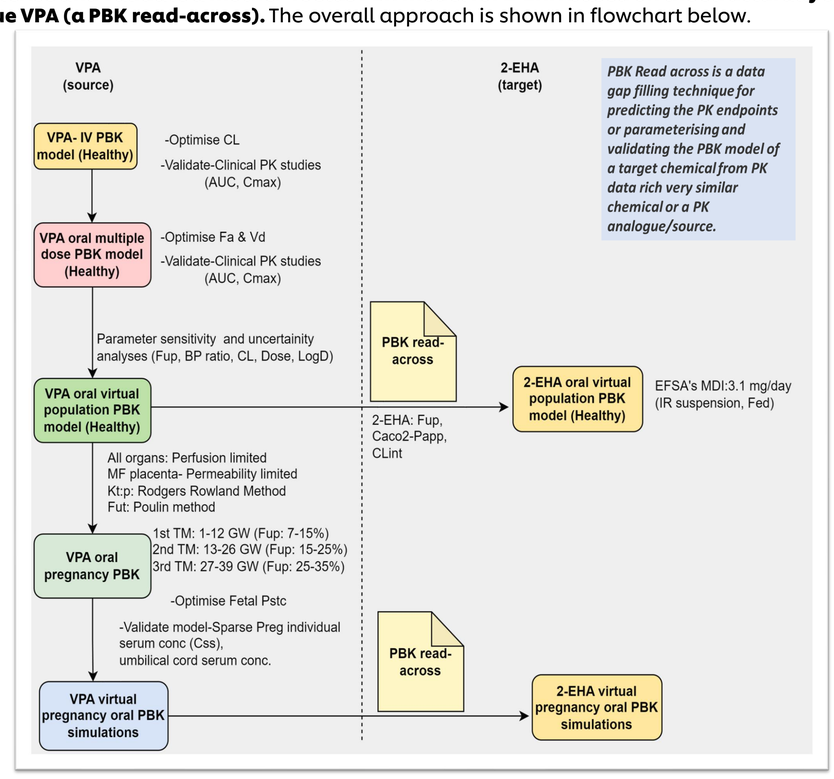


Fig.1: Flowchart for PBK-read across between VPA and 2-EHA.

2. Methodology:

	VPA	Reference	2-EHA	Reference
Mol wt (g/mol)	144.21	PubChem	144.21	PubChem
Log P	2.75	PubChem	2.64	PubChem
Solubility (mg/ml)	1.35	DrugBank (FDA label)	1	PubChem
pKa (acid)	4.8	PubChem	4.8	PubChem
Fraction unbound in plasma (Fup)	7-15%	Cloyd et al., 2003	13.16%	JRC report
Blood to plasma ratio (B/P) ratio	0.55	Soars et al., 2002	0.55	Assumed (OECD report)
Human Caco-2 assay: Pappa (10E–4 cm/s)	0.22	Torii eta al., 2002	0.55	Wu et al., 2023
Human Intestinal effective permeability-Peff (10E–4 cm/s)	3.84	Conner et al., 2018	1.17	G+ predicted (QSAR method)
Clint (µl/min/10^6 cells)-Hepatocytes	0.9	Fortaner et al., 2021	0.6	experimental (HµREL coculture system) (OECD report)
CLrenal (L/hr)	0.0079	Conner et al., 2018	0.0079	Assumed

Table 1: Literature based phys. chem. and in vitro ADME parameters for VPA & 2-EHA.

Predicted or measured physico-chemical and in vitro ADME properties were extracted from the literature studies for VPA and 2-EHA (see table 1). These parameters are used to build non-pregnant VPA/2-EHA PBK model In GastroPlus®.

Pregnancy related ADME changes for VPA (see table 2) were also extracted from literature and used for building the Pregnancy VPA PBK model in GastroPlus®.

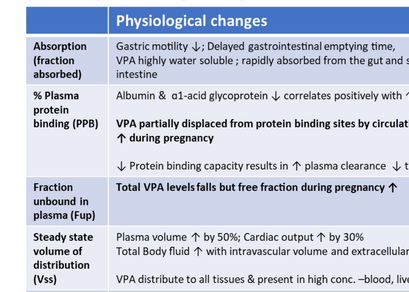
	Physiological changes	Values	Reference
Absorption (fraction absorbed)	Gastric motility ↓; Delayed gastrointestinal emptying time, VPA highly water soluble ; rapidly absorbed from the gut and sodium salt from the intestine	No change in pregnancy	Nau et al., 1981
% Plasma protein binding (PPB)	Albumin & α1-acid glycoprotein ↓ correlates positively with ↑ with gestational age **VPA partially displaced from protein binding sites by circulating free fatty acids which ↑ during pregnancy** ↓ Protein binding capacity results in ↑ plasma clearance ↓ total serum levels	Normal or 1st trimester (TM): 93-85% (Fup: 7-15%) 2nd TM: 85-80% (Fup: 15-25%) 3rd TM: 75-65% (Fup: 25-35%)	Nau et al., 1981
Fraction unbound in plasma (Fup)	**Total VPA levels falls but free fraction during pregnancy ↑**	**Serum protein binding of VPA is sig. ↓ in preg women upto 2 fold ↑ in free fraction**	
Steady state volume of distribution (Vss)	Plasma volume ↑ by 50%; Cardiac output ↑ by 30% Total Body fluid ↑ with intravascular volume and extracellular fluid VPA distribute to all tissues & present in high conc. –blood, liver and kidney	Vd ↑ with ↑ in extracellular fluid , fat content and expanding foetal compartment Apparent Vd 0.14-0.20 L/kg in adults	Nau et al., 1981
Clearance (CL)	↑ progesterone -↑ hepatic enzymes; estrogens are inhibitors (large interindividual variations due to varying ratios of hormones among the individual) ↑ renal clearance and metabolic capacity and ↑ in tissue binding	Clearance increased significantly (approx. 3 times) from 1ST TM to 3rd TM	Koerner et al., 1989

Table 2: Pregnancy related ADME changes for VPA.

Parameters incorporated for pregnant and foetal PBK modelling:

- Based on Nau (1981) plasma protein binding (PPB) or Fraction unbound in plasma (Fup of VPA changes throughout pregnancy. 1st Trimester (TM): 93-85% (Fup- 7-15%); 2nd TM: 85-80% (Fup-15-25%); 3rd TM: 75-65% (Fup-25-35%) was considered. As a result, the systemic clearance (CL) and volume of distribution (Vd) calculated in the PBK model (GastroPlus®) also changed.
- For the VPA/2-EHA pregnancy model, a placental permeability limited tissue model was applied, and the estimated permeability surface area product (Pstc) of 15000 ml/s was used to capture the foetal plasma concentration profile.
- The VPA Pregnancy model was validated against observed individual maternal serum and foetal cord blood concentrations.
- For the PBK read across from VPA to 2-EHA, for 2-EHA specific Fup, Caco2 assay based apparent permeability (Papp) and hepatocytes intrinsic clearance (CLint) values were changed.
- For the 2-EHA virtual pregnancy population PBK model, Fup range of 7-35% was incorporated by changing the % coefficient of variance (CV) to 23 to account for the interindividual variabilities in % PPB based on VPA pregnancy model.

3. Results:



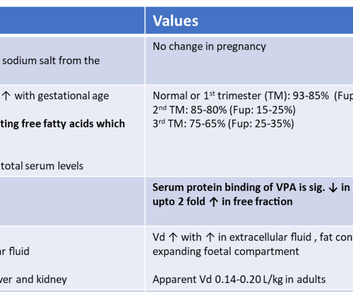


Fig.2: Parameter sensitivity and uncertainty analysis A) Fup vs Cmax B) Sensitivity index for all the critical parameters; Note: RBP: Blood to plasma ratio; Kp: tissue plasma partition coefficient

Uncertainty Ratio = 95th Percentile / Median

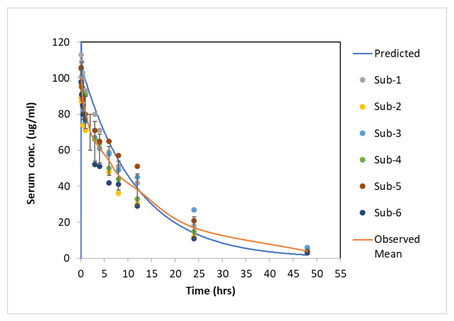
	Uncertainty ratio	
B:P ratio	1.0	Medium
Fup(%)	3.0	High
CL	1.2	Medium
Dose	2	High
LogD	1.7	Medium

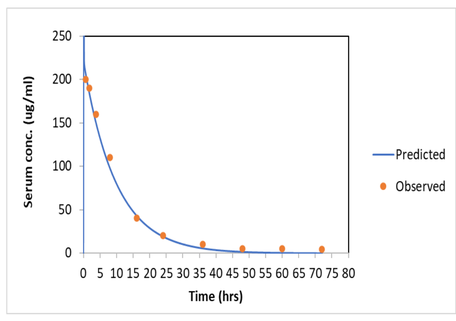
– high uncertainty: factor ≥ 2
– medium uncertainty: 0.3<factor<2
– low uncertainty: factor≤ 0.3

Table 3: Uncertainty ratios and classification for 2-EHA

Parameter sensitivity analysis found Fup to have the biggest influence on simulation outputs towards the Cmax during pregnancy (Fig 2A/2B). Fup and dose shows highest uncertainty for the virtual pregnancy model (Table 3). The results confirmed that Fup is the critical parameter for the target (2-EHA) compound for which probability distribution range needs to be considered to account for the interindividual variabilities in both the virtual non-pregnant and pregnant population.

A) Nitsche et al., 1982 (IV 1000 mg) **B)** Georgoff et al., 2017 (IV 2151 mg) **C)** Nitsche et al., 1982 (Multiple Oral dose- 900 mg/12 hrs)





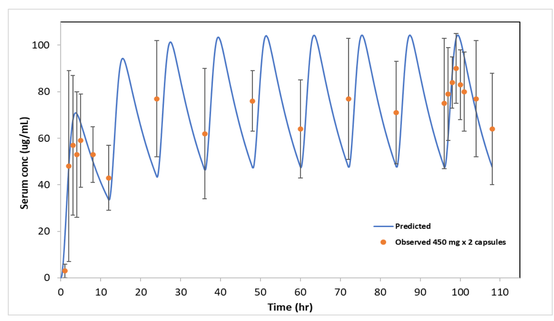
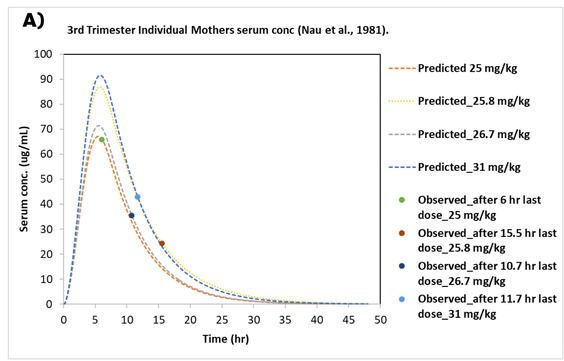


Fig.3: Comparison of VPA healthy volunteer PBK model predictions with clinical data. A healthy VPA PBK model was created using input parameters from table 1 and compared to clinical data for single IV exposure (A,B) as well as multiple oral dosage (C).

A healthy volunteer VPA PBK model was created and compared to outcomes form clinical studies. The model predicted the observed AUC and Cmax seen in clinical studies very well (see Fig.3) with a total fold error within 0.9-2.3-fold. Similar results were seen for comparing pregnancy VPA PBK models with maternal serum concentrations in 1st and 2nd trimesters which are in good agreement with the observed clinical outcomes and are below a fold error of 2 (data not shown).

A)



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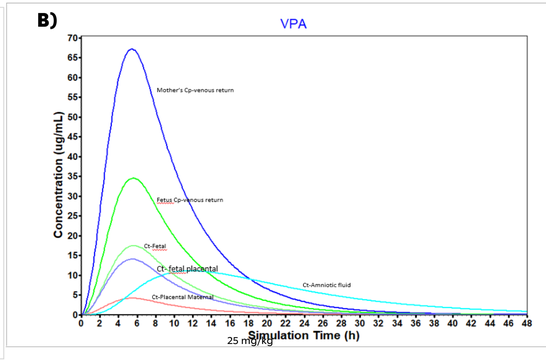


C)

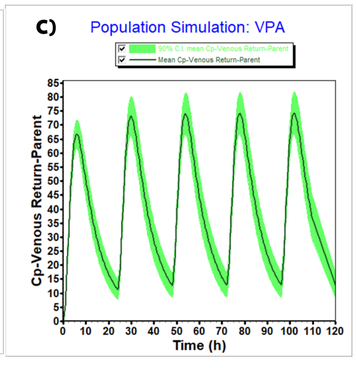


Fig.4: PBK model for VPA for maternal, foetal and population exposure A) An individual mother's predicted serum PK profile (at the time of delivery) superimposed with a single observed serum concentrations at various doses from Nau et al., 1981 study. B) A representative Gastroplus predicted mother and foetus (plasma, placental and amniotic fluids) full PK profiles at 25 mg/kg dose. C) Multiple dose mother's serum simulation output for VPA virtual pregnancy population.

A VPA pregnancy PBK model was created using GastroPlus which show good overlap with observed individual mother's serum concentration (Css) (see Fig.4 A) However this model underpredicted the foetal cord serum conc. (see Fig.4 A and table 4) estimating a foetal cord serum: mother serum (FM) ratio-approx. of 1 where the observed FM ratio is much higher in vivo (see table 4).

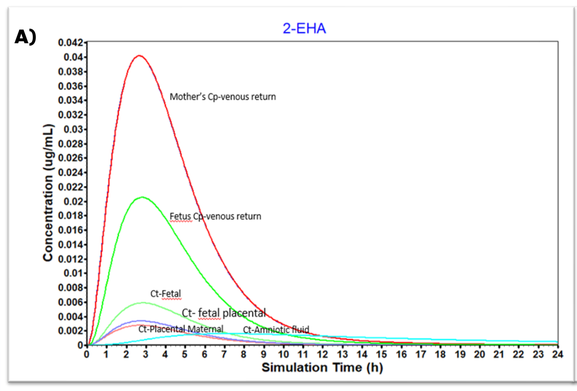
Dose (mg/kg)	Time of blood collection after last dose (hr)	Obs Mother serum (µg/mL)	Pred Mother serum (µg/mL)	Fold error (Pred mother serum ÷Obs mother serum conc.)	Obs Cord serum conc. (µg/mL)	Pred: Cp-Fetal Arterial or venous Supply (µg/mL)	Fold error (Pred Cp- fetal arterial or venous ÷ Obs cord serum conc.)	Obs FM ratio	Pred FM ratio (Pred Cp- fetal arterial or venous ÷ Pred mother serum conc.)
31	11.7	43.2	42.9	0.99	70.5	22.7	0.32	1.63	0.53
25	6	62.7	65.8	1.05	88	34.2	0.39	1.4	0.52
26.7	10.7	27.1	35.6	1.31	80	19	0.24	2.95	0.53
25.8	15.5	25.1	24.3	0.97	31	13.0	0.42	1.23	0.53

Note: Individual Fup considered, 31 mg/kg=30; 25 mg/kg=35; 26.7 mg/kg=35; 25.8 mg/kg=25.

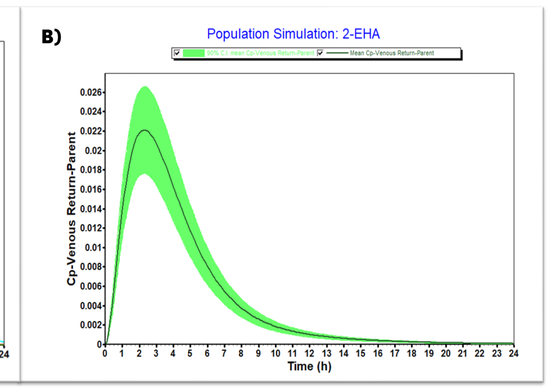
Table 4: Measured and predicted maternal serum and foetal cord serum concentrations for VPA.

Using read across a PBK model can be built for 2-EHA that allows reliable predictions to be made for healthy/non-pregnant and maternal Cmax values. Predictions for foetal exposures are less reliable as the PBK model for VPA underpredicted the observed FM ratio. These Cmax calculations will be useful for comparing with *in vitro* Point of departure (POD) values to derive a Bioactivity Exposure Ratios (BERs) which can be used in a Next generation risk assessment (NGRA) approach.

A)



B)



	Predicted Conc. (uM)
Mother Cp-Venous Return	0.28
Cp-Fetal Arterial Supply	0.14
Cp-Fetal Venous Return	0.14
Ct-AmnioticFluid	0.01
Ct-Fetal	0.04
Ct-PlacentaFetal	0.02
Ct-PlacentaMaternal	0.02

Fig.5: PBK model for 2-EHA A) Predicted mothers and foetus (plasma, placental and amniotic fluids) full PK profiles at 3.1 mg/day dose. B) A single dose mother's serum simulation output for 2-EHA in a virtual pregnancy population.

Table 5: Predicted maternal serum and foetal cord serum concentrations for 2-EHA.

4. Challenges:

a. For robust PBK read across, the source chemical should have full PK profiles in the non-pregnant and ideally pregnant population. However, the ethical concerns associated with studies that sample placenta, fetal organs, or systemic circulation during pregnancy for PK will always mean that published datasets in this space will be scarce. Likewise, levels of fetal drug exposure remain difficult to quantify from published information, as data from the fetus and placenta will always be limited to post-delivery measurements.

b. In the pregnancy PBK read across, only partial validation of the predicted mother's serum or foetus cord serum is feasible. Published data on fetal and maternal plasma ratio measurements are challenging to use in this work, as only one sample can be obtained per subject within a short time frame and the sampling time is relative to the last maternal dose taken.

c. The PBK predictions of foetal exposure to chemicals could be improved by better understanding and parameterisation of placental transfer including accounting for the abundance of placental enzymes and the interplay of influx or efflux transporters on the basolateral membrane of placenta. E.g., literature studies indicated the role of proton dependent monocarboxylic transporters in the transport of ionized VPA from mother to foetus compartment (Ishiguro et al., 2018).

d. For better foetus exposure predictions, further optimisation, and incorporation of measured blood to placental partitioning coefficient (Kp) and placental permeability surface area product (PStc) in the Pregnancy PBK model is required.

5. Conclusions:

This PBK read across assisted in the mechanistic understanding of the ADME processes of 2-EHA i.e., the identification and assigning the uncertainty of the most sensitive physiological parameter- the Fup for the pregnant population. This allowed building some confidence in the target chemical's simulation outputs based on source chemical's clinical PK datasets.

6. Future studies:

To better predict the asymmetric distribution of VPA or 2-EHA across the mother and foetus compartment, advanced cellular test system to characterise the kinetics of placental transfer, e.g. placenta-on-chip models or cell line-based models (such as BeWo) transfected with the transporters should be developed.

7. References:

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