Biokinetic and PBPK Modelling to Support Read-Across Assessment of Repeat Dose and Developmental and Reproductive Toxicity of Valproic Acid Analogues

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Workflow Overview

Physiologically based pharmacokinetic (PBPK) models and biokinetic models were used to support two read-across case studies assessing the toxicity of the valproic acid (VPA) analogues. Case study 1 (CS1) characterised the hazard of 2-ethylbutyric acid (2-EBA), specifically to induce hepatic steatosis, after 90 days repeated exposure. Case study 2 (CS2) characterised the developmental and reproductive toxicity (DART) hazard of 2-Methyl-hexanoic acid (2-MHA). Given the overlap in the target compounds (TC) and source compounds (SC), and availability of data for model verification, a uniform PBPK modelling strategy was developed using VPA as an exemplar compound.

- A rat PBPK model for VPA was developed using a reverse translation approach and used to select a nominal treatment concentration range for *in vitro* testing in CS1
- A feto-placental model was implemented in mouse and verified against *in vivo* study data and used to determine a fetal to maternal concentration ratio in CS2; in the absence of data for other SCs and the TC, this ratio (≈0.5) was assumed to be constant across the range of compounds investigated.
- The virtual in vitro distribution (VIVD) model (Fisher et al. 2018) was used to predict the intracellular concentrations achieved in *in vitro* assays given nominal treatment concentrations. Where this model could not be applied, unbound treatment medium concentrations were predicted using an alternative approach.
- Human PBPK models were developed using an *in vitro* to *in vivo* extrapolation (IVIVE) approach using *de novo* hepatic intrinsic clearance (CLi_{ntH}) and data recovered from the literature or predicted using QSAR models. Mouse PBPK models were parameterised based on allometric scaling of *in vivo* clearance predicted using human PBPK models.
- A reverse dosimetry approach was used to predict oral equivalent doses (OED; mg/kg) of TCs and SCs that would achieve the *in vitro* effective concentrations, corrected using biokinetic modelling, in target tissues *in vivo*.

Rat PBPK – Dose Selection for *In Vitro* Testing

A rat PBPK model describing the kinetics of VPA was developed using a reverse-translation approach and verified against legacy study data . The rat shows significant enterohepatic recirculation (EHR) after dosing with VPA (Dickinson *et al*. 1979). More specifically, biliary excreted, glucoronidated metabolites are deconjugated in the lumen of the gastrointestinal tract, resulting in the reabsorption of the regenerated parent compound. A semi-mechanistic model of deconjugation in the gastrointestinal tract of biliary cleared metabolites was implemented in the Simcyp Animal simulator (v17r1). Incorporation of this mechanism resulted in better recovery of the pharmacokinetic profile of VPA in the rat (figure 1). This verified model incorporating EHR was used to guide the selection of a concentration range for *in vitro* testing for CS1, translating established LOAEL doses to unbound plasma concentrations. Rat *in vivo* studies, using intraperitoneal and oral dosing, have determined a LOAEL (hepatic steatosis) of 500mg/kg. An oral repeat dosing study (500mg/kg, τ=24h; Abdel-Dayem, Elmarakby et al. 2014) was simulated. Based on this a maximum unbound plasma concentration of ~2.5mM was identified as corresponding to the LOAEL established *in vivo* (table 1). *In vitro* studies, using the Huh7 human hepatocyte cell line, have previously shown accumulation of lipid droplets after 24hr treatment with 0.5mM VPA (Elphick, Pawolleck et al. 2012).

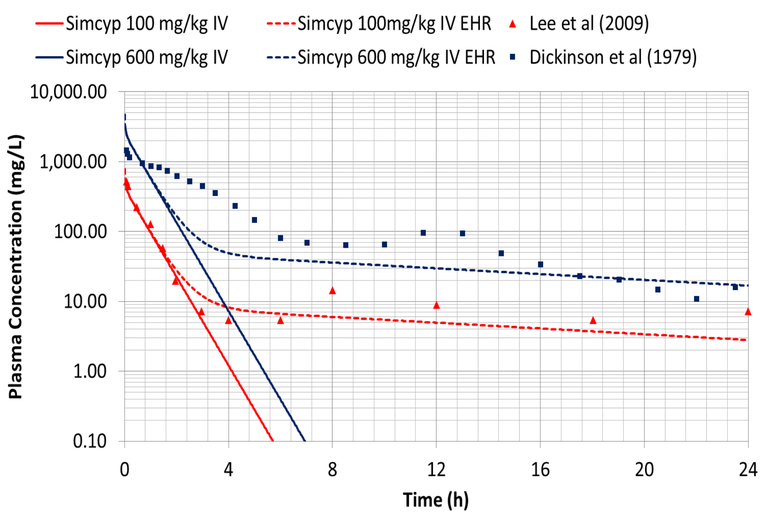


Figure 1. Plasma concentration-time profiles following intravenous (iv) dosing in the rat at 100mg/kg (red) and 600 mg/kg (blue). Simulations not accounting for enterohepatic recirculation (EHR) (solid lines) and accounting for EHR (dashed lines) are shown. Observed data from the in vivo dosing studies simulated are plotted as data points.

Table 1. Results from simulations of the repeat oral dosing study (500mg/kg, τ=24h; Abdel-Dayem, Elmarakby et al. 2014) using the verified rat VPA PBPK model.

Dose (mg/kg)	Plasma Unbound C_{max} (mM)	Plasma AUC (mg/L.h)	Liver Unbound C_{max} (mM)	Liver AUC (mg/L.h)
500	2.51	2184.8	2.52	407.3

Prediction of *In vitro* Biokinetics

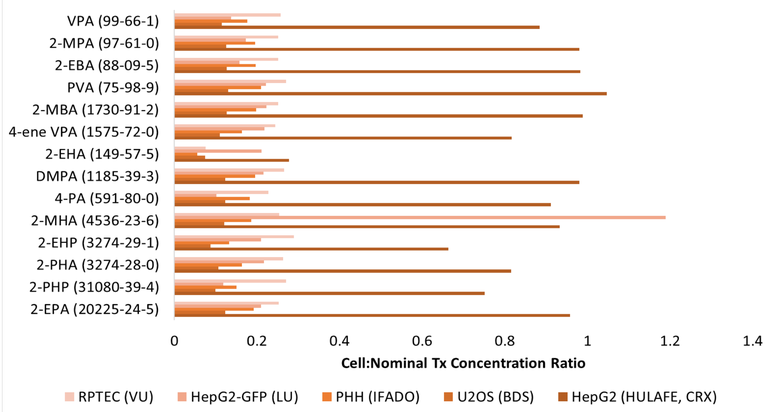


Figure 2. Predicted cell to nominal treatment concentration ratios based on compound physicochemical properties, cell composition and assay conditions using the VIVD model.

$$fu_{media} = \frac{1}{1 + K_{protein} f_{protein} + \frac{P_{nl} f_{nl}}{1 + 10^{(pH - pKa)}}}$$

$$f_{nl} \approx f_{TAG} = \frac{[TAG] \cdot PSV_{TAG}}{1000}$$

$$f_{albumin} = \frac{[albumin] \cdot PSV_{albumin}}{1000}$$

Equation 1. Equations used to determine the fraction of unbound test compound in treatment medium (fu_{media}) based on the composition of medium in terms of neutral lipid (nl), specifically triacylglycerol (TAG) and albumin. Partitioning coefficients between medium and neutral lipid and medium and albumin, Pnl and Kprotein, respectively, were calculated based on physicochemical properties.

Nominal treatment concentrations applied to *in vitro* test systems do not necessarily translate to the concentration at the site of action. To improve the translation of the effective concentrations identified *in vitro* to *in vivo*, the VIVD biokinetic model (Fisher et al. 2018) was used to predict the ratio between the intracellular concentration and the nominal treatment concentration (figure 2). The VIVD model assumes steady-state conditions and as such this ratio can be applied as a constant, assay specific, correction factor to all nominal treatment concentrations applied. Where the assumptions of the VIVD model were not compatible with the *in vitro* assay set-up (e.g. UKN1 and mEST assays, CS2) a simplified approach was used to predict the unbound concentration of test compound in the treatment medium applied in the assay system (equation 1). Reverse dosimetry to determine OEDs was performed with respect to target tissue, where VIVD corrections had been applied, or alternatively to unbound plasma concentrations .

Modelling Assumptions Based on VPA Source Compound

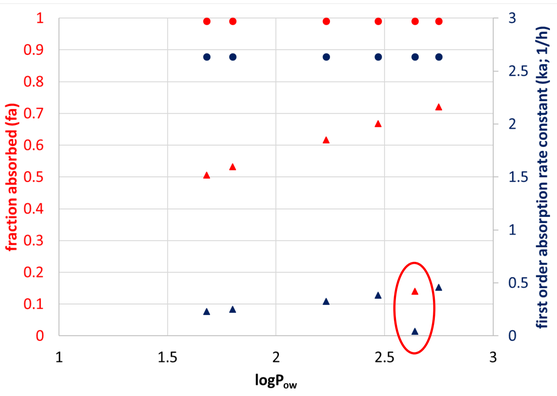
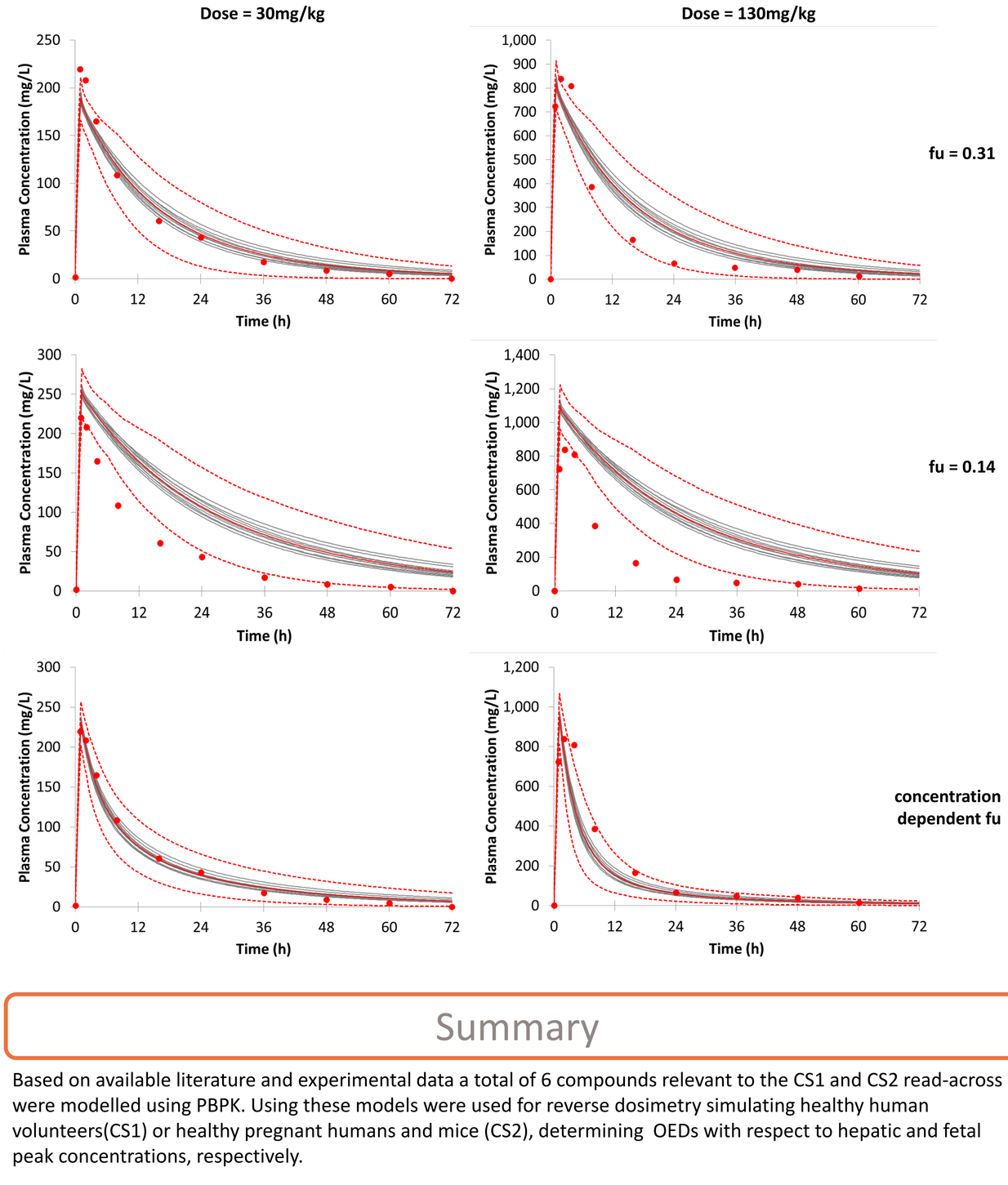


Figure 3. Comparison of the fraction absorbed (fa, red) and first order absorption rate constant (ka; 1/h, blue) predicted using the Simcyp QSAR model based on polar surface area (PSA, Å²) and hydrogen bond donors (HBD) (circles) or the MechPeff model (triangles). MechPeff predicted values for 2-Ethyl Hexanoic acid (2-EHA) are circled.

Given the structural similarity between the CS1 and CS2 TCs and identified SCs, predictions of fa and ka using the Simcyp QSAR model does not distinguish between the compounds for which PBPK models could be generated. The Simcyp MechPeff model predicts increasing fa and ka with increasing $logP_{ow}$, with the exception of 2-EHA due to a greater fraction ionised; 2-EHA has a pKa≈ 3.3, while the rest of the compound series have pKa ≈ 4.8 (figure 3). Since it was not possible to verify these model predictions, the MechPeff model was applied in mouse PBPK models, to more mechanistically predict oral absorption in the preclinical species. In contrast, the Simcyp QSAR model was applied in human PBPK models to predict fa and ka. As such, simulations with the human PBPK model assume that following oral dosing, analogues investigated in these read-across are efficiently and rapidly absorbed, providing a prediction of the worst-case scenario following oral exposure.

Due to analytical issues, it was not possible to determine the fraction unbound in plasma (fu) experimentally. QSAR models for predicting fu were established. However, given that experimental data was only available for VPA, and VPA has been shown to demonstrate concentration dependent plasma protein binding (Ogungbenro, Aarons et al. 2014), it was not possible to select a single predictive model. A human VPA PBPK model was established using literature data and hepatic intrinsic clearance determined *in vitro* by Cyprotex using the HμREL co-culture system (Hurel Corp, NJ, USA) and three alternate assumptions were assessed with respect to fu; using the highest predicted, using the lowest predicted fu, or simulating concentration dependent protein binding (figure 4). While simulations with concentration dependent fu, best recovered the observed data, there was a tendency to under-predict exposures with increasing dose. This is due to the simulation of saturable plasma protein binding in conjunction with non-saturable metabolic clearance. The decision was made to perform reverse dosimetry with two PBPK models using the lowest and highest QSAR predicted fu, and so provide a range of predicted OEDs.

Figure 4. Human PBPK simulations of VPA plasma concentration time profiles using alternative fraction unbound in plasma values. 10 simualted trials (10 individuals per trial, proportion female 0.08); mean of individual trials (grey lines), mean of population (n=100, red line), 5th and 95th percentile of simulated population (dashed red lines), observed data (red data points, Georgoff et al. 2018)



Summary

Based on available literature and experimental data a total of 6 compounds relevant to the CS1 and CS2 read-across were modelled using PBPK. Using these models were used for reverse dosimetry simulating healthy human volunteers(CS1) or healthy pregnant humans and mice (CS2), determining OEDs with respect to hepatic and fetal peak concentrations, respectively.

Table 2. Summary of experimentally determined hepatic intrinsic clearance ($CL_{int,H}$), and Simcyp PBPK (v17r1) predicted in vivo clearance (CL_{iv})

	99-66-1 VPA	149-57-5 2-EHA	20225-24-5 2-EPA	88-09-5 2-EBA	97-61-0 2-MPA	4536-23-6 2-MHA
$CL_{int,H}$ (µl/min/10^6; HμREL co-culture)	0.22	0.55	0.78	9.62	10.2	3.95
CL_{iv} (L/h (human; predicted, min fu)	0.33	0.85	2.38	20.42	21.09	9.10
CL_{iv} (L/h) (human; predicted, max fu)	0.73	1.80	3.80	29.09	29.07	13.68
CL_{iv} (ml/min) (mouse; predicted, min fu)	0.01	0.03	*NA*	0.80	0.82	0.35
CL_{iv} (ml/min) (mouse; predicted max fu)	0.03	0.07	*NA*	1.13	1.13	0.53

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