Application of in vitro hepatic clearance data and physiologically based kinetic modeling for the estimation of species differences in internal exposure

Jochem Louisse*, Nicole Pinckaers, Karsten Beekmann, Ad Peijnenburg, Ans Punt
Wageningen Food Safety Research, Wageningen University and Research, Wageningen, The Netherlands

Background

- In vitro kinetic data are useful to provide insights into species-dependent differences in kinetics (and related internal exposure), when integrated in physiologically based kinetic (PBK) models.
- These insights can facilitate the translation of animal toxicity data to humans, e.g. by estimating the relative sensitivity of humans to the test species, related to possible differences in internal exposure.

Aim of the study

- Obtain in vitro-derived PBK model input data for rats and humans for 8 model compounds (see **Table 1**) related to:
  - Intrinsic hepatic clearance (CLint)
  - Fraction unbound plasma (fup)
- Predict differences in internal exposure (plasma Cmax and AUC) upon single or repeated (28 days) exposure to a fixed dose (0.1 mg/kg bw) with PBK modeling.

Methods

- In vitro hepatic CLint values were derived by measuring the time-dependent depletion of the parent chemical in in vitro incubations with rat or human liver S9 (from pooled donors) (**Figure 1**).
- Fup was determined for rat or human plasma using a rapid equilibrium dialysis device (Fisher Scientific) using different plasma dilutions (**Table 1**).
- LogP and pKa of chemicals were estimated with Chemicalize (**Table 1**).
- In vitro-derived chemical input data and information on chemical characteristics (LogP and pKa) were applied in our recently developed online generic PBK modelling platform (www.qivivetools.wur.nl) to predict internal exposure.

Results

in vitro substrate depletion with rat or human liver S9

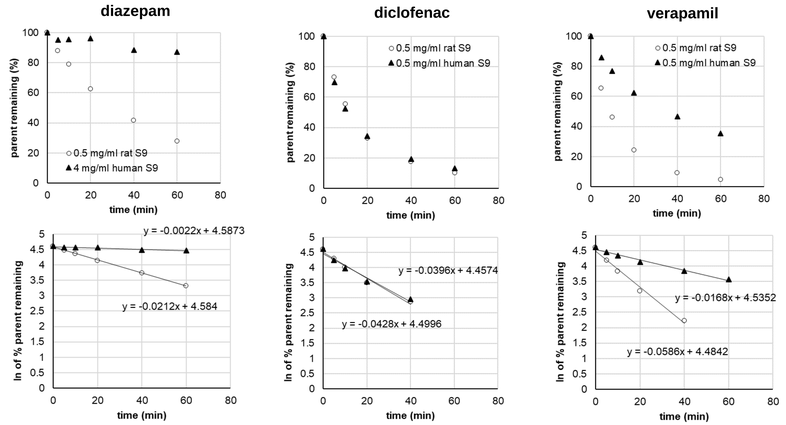

Figure 1. Example data of substrate depletion studies with diazepam, diclofenac and verapamil in incubations with human or rat liver S9 together with cofactors for major Phase I and Phase II reactions (NADPH, UDPGA, PAPS). For each combination of chemical and S9, optimal concentrations were determined. Full depletion curves for each combination of chemical and S9 type were obtained for using at least 2 different S9 concentrations (data for only one S9 concentration presented).

Table 1. Chemical-specific input data applied in the online generic PBK modelling platform (www.qivivetools.wur.nl). PBK model-based predictions of diazepam, diclofenac and verapamil plasma concentrations are presented in **Figure 2**. Differences in predicted internal exposure for all 8 chemicals are summarized in **Figure 3**.

chemical	charge	LogP [a]	pKa [a]		fup [b]		CLint,u (uL/min/mg S9) [c]		ka (hr^{-1}) [d]	
			acidic	basic	rat	human	rat	human	human	rat
dextromethorphan	base	3.49		9.9	0.33	0.39	285	24.1	2.87	4.43
diazepam	neutral	3.08			0.17	0.025	44.2	1.83	2.28	3.52
diclofenac	acid	4.26	4.0		0.0079	0.0039	91.8	144	1.89	2.92
midazolam	base	3.97		6.2	0.070	0.028	413	122	2.35	3.63
naloxone	base	1.48		7.9	0.58	0.57	284	15.9	1.5	2.31
propranolol	base	2.58		9.7	0.26	0.22	451	9.15	2.07	3.19
tolbutamide	acid	2.3	4.4		0.026	0.024	0.11 [e]	0.42 [e]	1.28	1.98
verapamil	base	5.04		9.7	0.13	0.15	420	178	1.6	2.47

a) Estimated with Chemicalize (https://chemicalize.com).
b) Determined using a rapid equilibrium dialysis approach applying different plasma concentrations. From incubations with acceptable recovery (90-110%), fup values were calculated.
c) Estimated based on the CLint obtained from the substrate depletion data, corrected for unspecific protein binding (estimated using the method of Hallifax and Houston (2006; www.doi.org/10.1124/dmd.105.007658) for microsomes.
d) Estimated based on topological surface area (obtained from Pubchem) as described in Punt et al. (2020; www.doi.org/10.1021/acs.chemrestox.0c00307).
e) Limited depletion of tolbutamide, providing possibly unreliable CLint values.

PBK model predictions obtained with www.qivivetools.wur.nl

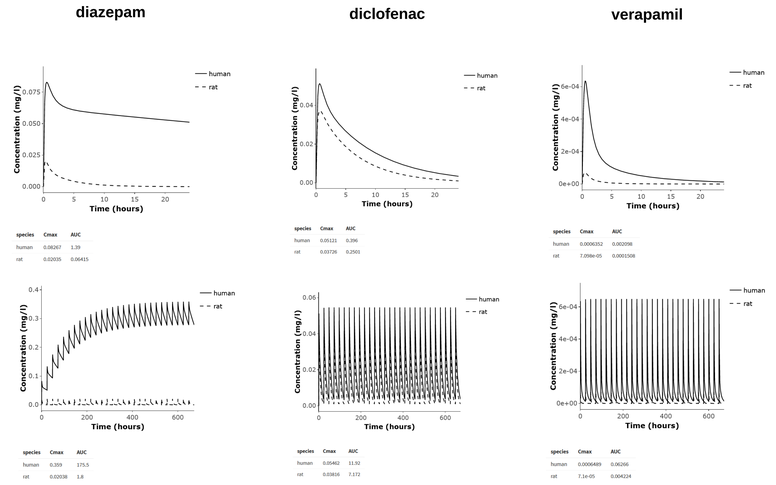

Figure 2. PBK model predictions of internal dosimetry of diazepam, diclofenac and verapamil. Chemical-specific input data as presented in **Table 1** were applied in our online generic PBK modelling platform (www.qivivetools.wur.nl). Screenshots of the PBK model output (plasma concentrations) from the online platform are presented for single or repeated (28 days) exposure to 0.1 mg/kg bw/day, also presenting the obtained Cmax and AUC.

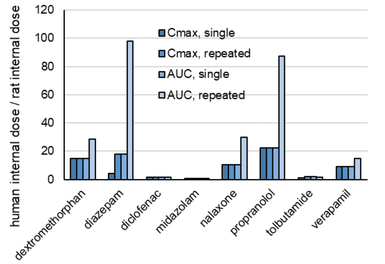

Figure 3. Ratio of PBK model-predicted internal dose in humans to PBK model-predicted internal dose in rats regarding Cmax and AUC in plasma upon single or repeated (28 days) dosing to 0.1 mg/kg bw/day.

Main findings and further steps

Differences in internal exposure were predicted for five chemicals. For such chemicals, more detailed analyses may be warranted when extrapolating rat toxicity data to humans. PBK model predictions will be evaluated against in vivo data to provide more insight into the applicability domain of the approach, and the possible need of including other kinetic processes in the generic PBK model.

*contact: jochem.louisse@wur.nl

Acknowledgements: this work was funded by the Dutch Ministry of Agriculture, Nature and Food Quality (project WOT-02-002-003)