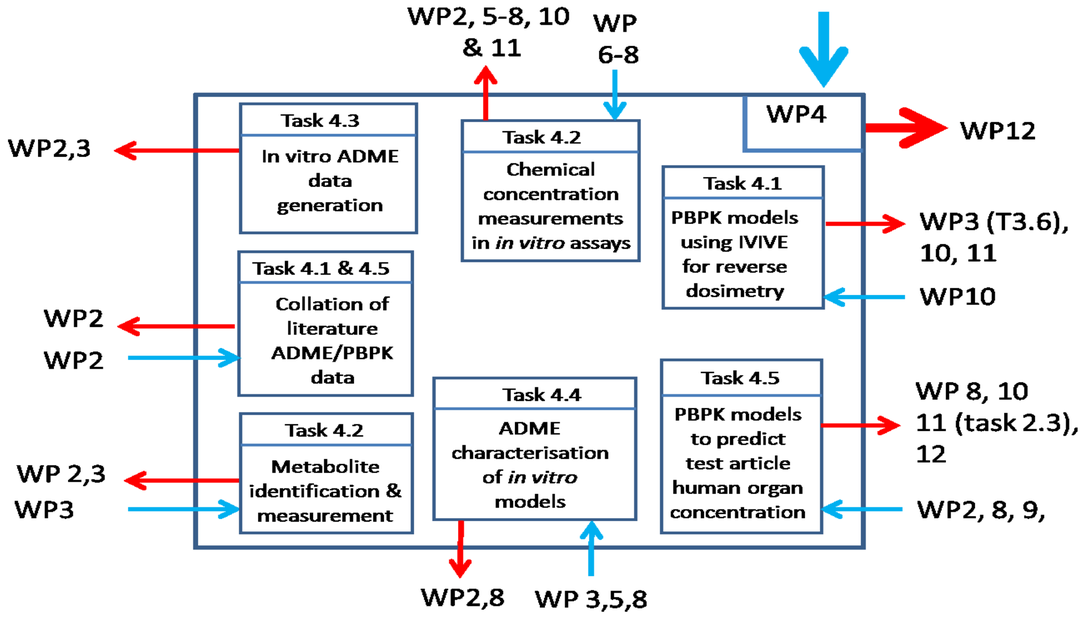
WP4 – In Vitro/In Silico Biokinetics, ADME and Physiologically Based Pharmacokinetic (PBPK) Modelling

Objectives

1) To establish the relationship between applied chemical dose and intra and extra cellular concentrations in the *in vitro* test systems
2) To predict the concentrations achieved in the different organs of the human body following *in vivo* exposure to chemicals
3) To generate *in vitro* human ADME data to help build and test *in silico* models
4) To characterize *in vitro* model systems for drug disposition protein expression and activity

Connectivity with other work packages



Physiologically based Pharmacokinetic (PBPK) models

PBPK models have been constructed to allow the prediction of human pharmacokinetics of compounds in CS 1, 2, 3, 4, and 6. Multi-compartment PBPK models have been developed for the placenta, liver and lung to help with the various case studies (see figure 3 and separate posters)

To help parameterize the model and to allow comparisons with the simulated results available pharmacokinetic data has been collated and curated to ensure a high-quality dataset is available (see Figure 1, bekow)

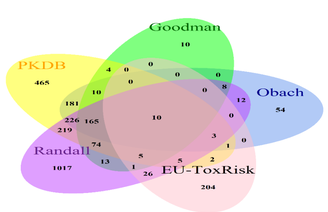


Figure 1. Venn diagram showing availability of PK data for the EU-ToxRisk case-study chemicals in individual pharmacokinetics databases: PKDB, Randall, Goodman and Gilman, and Obach.

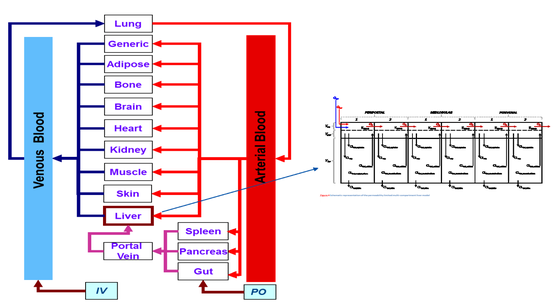


Figure 2. Schematic diagram of the multi compartment liver model showing the processes that can be modelled in each of 6 zones within the liver model. The model allows active (CL,total uptake) and passive movement of drug into the liver as well as metabolism and efflux either to the bile or blood to be accounted for.

CAS	compound	PR-antI	TRb	PXR	PPARa	TCF	ESRE
99-66-1	Valproic Acid	-4.0	-3.9	-5.0	-4.9	-4.9	-4.9
	VPA (uM)	91.58	115.29	9.16	11.53	11.53	11.53

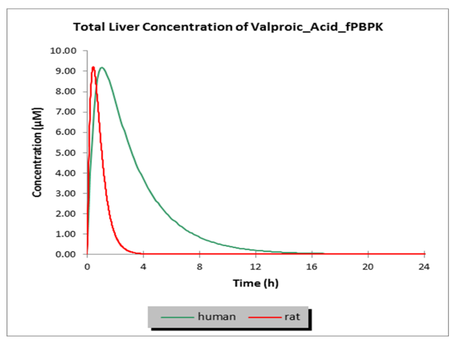


Figure 3 PBPK model simulated valproic acid liver concentration in rat and human at doses that achieve the same concentrations in the liver as the lowest concentrations that activate liver receptors in the BDS *in vitro* receptor assays. The doses achieving this concentration were 4 mg/kg in rat and 1.75 mg/kg in human. These doses are in the range of clinically used valproic acid doses but are significantly lower than the LOAEL in rats (500 mg/kg). This suggests that the MIE are triggered at much lower doses than LOAEL in both species.

Biokinetic and QSAR modelling

Biokinetic models have been developed to enable the intracellular concentration of drugs to be predicted. The developed model accounts for binding to plastic, intra and extracellular proteins and lipids as well as the volatility of the chemical. The effect of cell membrane potential and the differential distribution of chemicals into cellular organelles is also considered. The initial static model has been extended to account for dynamic processes such as metabolism and cell growth (see separate posters)

QSAR models for some of the ADME data such as protein binding and PGP/BCRP affinity have been developed in collaboration with WP3 (see separate posters)

Generation of *in vitro* ADME data

To build PBPK models for compounds a number of input parameters are needed. These include plasma protein binding, blood:plasma ratio, an estimate of metabolic stability in human hepatocytes, solubility and permeability measurements. This data is being generated for case study compounds by WP4.

For some compounds the ability of drug transporters to alter the intracellular concentration needs to be considered. Medium and high throughput assays have been established to to measure the activity of P-gp and BCRP, two important drug efflux transporters.

ADME characterisation of *in vitro* models

The expression of 291 relevant drug metabolising enzymes and transporters in the different *in vitro* systems available to the project has been investigated using mRNA expression approaches. The results are in the process of being analysed currently (Figure 4)

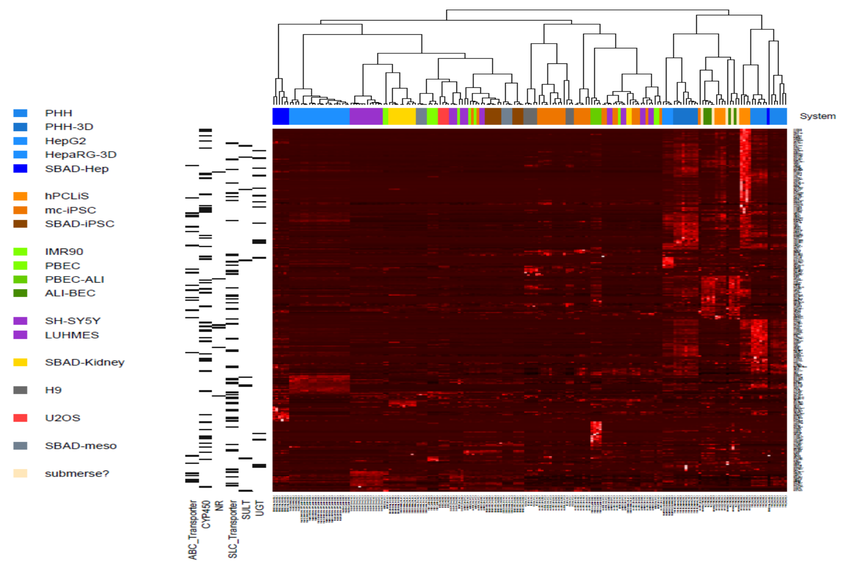


Figure 4 Heat map showing the expression of different drug metabolising enzymes, transporters and nuclear receptors in the different *in vitro* systems available within the EUTOXRISK project. The level of expression of a particular enzyme transporter is shown by the shade of red with black being no expression increasing from dark red to bright red and white as the highest expression.

The EU-ToxRisk project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002.

www.eu-toxrisk.eu/