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Abstract

Physiologically-based toxicokinetic (PBTK) models are important tools for *in vitro* to *in vivo* or inter-species extrapolations in health risk assessment of foodborne and non-foodborne chemicals. Here we present a generic PBTK model implemented in the EuroMix toolbox, MCRA 9 and predict internal kinetics of nine chemicals (three endocrine disrupters, three liver steatosis inducers, and three developmental toxicants), in data-rich and data-poor conditions, when increasingly complex levels of parametrization are applied. At the first stage, only QSAR models were used to determine substance-specific parameters, then some parameter values were refined by estimates from substance-specific or high-throughput *in vitro* experiments. At the last stage, elimination or absorption parameters were calibrated based on available *in vivo* kinetic data. The results illustrate that parametrization plays a capital role in the output of the PBTK model, as it can change how chemicals are prioritized based on internal concentration factors. In data-poor situations, estimates can be far from observed values. In many cases of chronic exposure, the PBTK model can be summarized by an external to internal dose factor, and interspecies concentration factors can be used to perform interspecies extrapolation. We finally discuss the implementation and use of the model in the MCRA risk assessment platform.

Keywords: Physiologically-Based ToxicoKinetic (PBTK) model; risk assessment; mixtures; probabilistic model.

1 INTRODUCTION

Human health risk assessment relies upon understanding and estimating how a given exposure to xenobiotics can cause health hazards. Toxicokinetics bridge the gap between exposure to xenobiotics and internal concentrations that condition health hazards, by providing predictions of compounds' internal levels at target organs (i.e. where the toxic action occurs), which is particularly relevant for those compounds showing bioaccumulation potential.

Toxicokinetics can be modelled and predicted by Physiologically-Based Toxicokinetic (PBTK) models. PBTK models represent the organism as a set of compartments linked together by the bloodstream. Single-substance PBTK models predict the time-courses of the chemical inside each compartment that result from one or more user-defined exposures to a given chemical. These models can be used to compare kinetics of substances within a cumulative assessment group, which includes the substances for which an assessment of combined exposures is warranted, and therefore help identifying the drivers of internal exposure or toxicity in a mixture. The use of kinetic modeling in the higher tier of risk assessment of multiple substances has been recently recommended in order to improve hazard identification and characterization [1]. Chemical risk assessment increasingly calls for PBTK models when available [2–5], and, therefore, a need for tools available to regulators and stakeholders is also rising [6]. Over the past years, many tools have been developed for various applications including in a regulatory context [7]. For example, MERLIN-Expo simulates the fate of substances in environmental systems and in the human body [8,9]; *httk* provides a generic PBPK model and a large *in vitro* database as an R package [10].

During the EuroMix project, a module for kinetic models was developed and added to the Monte Carlo Risk Assessment (MCRA) platform for chemical risk assessment [11,12]. MCRA uses probabilistic methods to address all areas of risk assessment for combined

exposures to multiple chemicals and can be used for hazard identification, hazard characterization, exposure assessment and risk characterization [12]. The kinetic module includes a generic PBTK model designed to be well-suited for a wide range of chemicals, for which different parameterizations can be uploaded using data files. Kinetic models are available for both humans and rats with the same underlying structure but with species-specific physiological parameters. In the toolbox, kinetic models can be used to convert internal doses at the target organ (either in vitro exposure doses or rat internal doses) to human external exposure doses and vice versa. This dose conversion is used when performing *in vitro* to *in vivo* extrapolation (IVIVE) or animal to human extrapolation [12].

Building a PBTK model is challenging and requires gathering a considerable amount of data which can be categorized in three groups, namely, (i) the model structure, which refers to the arrangement of tissues and organs included in the model; (ii) the biological system's data (physiological, anatomical, biochemical data); and (iii) chemical-specific data (physicochemical). Chemical-specific data can be collected from a variety of sources, using default values, *in vivo* measurements, *in vitro* cell-based assays, or *in silico* predictions. Previously, PBPK models have been parameterized based first on *in vivo* data, with *in vitro* data added for various extrapolations between exposures or species [13], but for many substances, such as pesticides and contaminants, *in vivo* data in humans and even in rats are sparse or unavailable. Furthermore, use of non-animal data in PBPK modelling for risk assessment is being encouraged with the aim to reduce, and eventually replace, animal testing [13,14]. The amount of high-throughput *in vitro* data available nowadays is rapidly increasing, in particular regarding metabolism and chemical binding [10]. PBTK models are thus also increasingly used as screening tools to predict the potential for accumulation in tissues by integrating high-throughput *in vitro* data and *in silico* data [10].

Integration of PBTK models in a toolbox with such a broad spectrum as MCRA implies that they will be used, and parameterized, by end-users who may not be specialists. Guidance on the selection of appropriate parameters is scarce [6], but can greatly affect the quality of the model outcomes. This paper shows how increasing amounts of data can be used to refine PBTK model predictions as in a tiered approach [15] and which level of accuracy to expect in data-poor situations. We wish to draw attention to the fact that the underlying model structure, the default assumptions and the choice of user-defined parameters may have a large impact on the outcome of the risk assessment, especially when the kinetic module is used for structurally and functionally diverse substances. In this paper, we compare various levels of refinement in PBTK model parameterization, using QSAR models and *in vitro* data. We illustrate how these models can be used in cumulative risk assessment and what can be expected in terms of accuracy and uncertainty of the predictions. First, we examine a data-rich case, with valproic acid, which can cause cranio-facial malformations in foetuses whose mothers are exposed. Secondly, we extend the approach to eight other chemicals with varying amounts of available information on kinetics. These chemicals have been associated with three adverse outcomes in toxicological studies, i.e. steatosis (imazalil, thiacloprid, and clothianidin), endocrine effects (flutamide, linuron, and dienestrol), or cranio-facial malformations (cyproconazole, valproic acid, and triadimefon).

2 MATERIAL AND METHODS

2.1 PBTK model

2.1.1 Model description

The generic PBTK model implemented in MCRA is an updated version of a PBTK model developed by INERIS and JRC in the framework of the EU project COSMOS (part of the SEURAT-1 cluster, http://www.cosmostox.eu/ [3,4]). The original (unpublished) model was updated to improve the dermal absorption model, to impose correlations in the chemical tissues' affinities (i.e., tissue:blood partition coefficients) , and to allow more time- and dose-varying exposure patterns (Figure 1). The EuroMix generic PBTK model describes the distribution of chemicals in venous and arterial blood, adipose tissues, poorly perfused tissues (muscles), liver, richly perfused tissues (other viscera), and skin. Each of those is described as a compartment (homogeneous virtual volume) in which distribution is instantaneous and limited only by the incoming blood flow or rate of entry in the compartment [18]. Exposure can occur through the dermal route, ingestion or inhalation. The absorbed substances can be excreted to urine, exhaled through the lung, or metabolized in the liver. External compartments, including urine and gut lumen, are not modelled. The model is coded as a set of ordinary differential equations. There is one such equation per time-dependent chemical quantity of the model (so-called state variable). The model predicts, as a function of time, for given oral, dermal and/or inhalation exposures, the quantities in organs and the corresponding concentrations as a function of time. The model equations are provided in Supplementary Information (SI), section 1.1. The model, written in GNU MCSim [19], can be downloaded from ZENODO with the DOI 10.5281/zenodo.3553690 together with an example of code for running it under R [20].

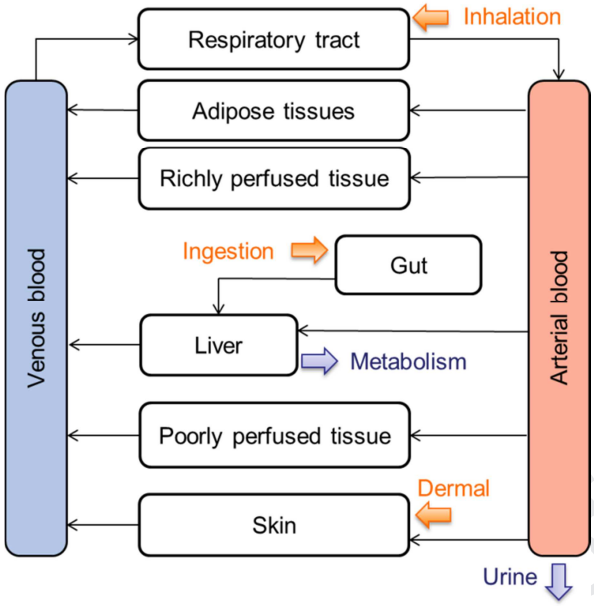

Figure 1: Schematic representation of the EuroMix generic PBTK model implemented in the MCRA platform.

2.1.2 Physiological parameter values

The PBTK model contains 14 physiological parameters. To model known correlations between parameter values, or to respect physical constraints in case of Monte Carlo sampling, some of them are scaled prior to solving the differential equations, using proportionality constants, in particular for relative tissue volumes and blood flows. Default mean values for physiological scaling coefficients and unscaled parameters for humans and rats (Table 1) have been collected from the literature.

Table 1: Physiological parameter values used for the Euromix generic PBTK model

Parameter	Symbol	Units	Human		Rat	
			Value	Ref	Value	Ref
Body mass (BM)	*BM*	kg	70	[21]	0.3	[22,23]
Relative tissue volumes (% BM)						
Fat	*scVFat*	-	20.9	[22,23]	7.3	[22,23]
Richly perfused	*scVRich*	-	10.5	[22,23]	10.0	[22,23]
Liver	*scVLiver*	-	2.4	[22,23]	3.5	[22,23]
Blood	*scVBlood*	-	6.8	[21]	6.8	[21]
Cardiac Output (CO)	*scFBlood*	L/h/kg	4.8	[21]	18.8	[21]
Relative tissue blood flows (% CO)						
Fat	*scFFat*	-	4.6	[22,23]	0.54	[22,23]
Poorly perfused	*scFPoor*	-	13.4	[22,23]	10.0	[22,23]
Liver	*scFLiver*	-	25.9	[22,23]	16.0	[22,23]
Skin	*scFSkin*	-	5.4	[22,23]	7.8	[22,23]
Alveolar ventilation rate	*Falv*	L/h	2220	[23]	6.35	[23]
Stratum corneum thickness	*Height_sc*	dm	0.0001	[24]	0.0001	[24]
Body skin surface area	*BSA*	dm^2	190[a]	[24]	3.64[a]	[25]
Viable skin thickness	*Height_vs*	dm	0.0122[b]	[22,23]	0.0094[b]	[22,23]

[a] Here a default value is used. In MCRA, when performing aggregate exposure assessment, the BSA is scaled to the bodyweight using an allometric scaling factor, it is not rescaled in hazard calculations as in this application.

[b] Value obtained by dividing the skin volume by the body surface area.

2.1.3 Parameterization of the substance-specific values

The PBTK model contains up to 14 substance-specific parameters (see list of parameters in SI, section 1.2, Table 1), as absorption rates, partition coefficients, or hepatic clearances. Metabolism was always modelled as first-order rather than Michaelis-Menten kinetics which decreases the number of substance-specific parameters to 13. Chemical-specific parameters can be obtained from *in vivo* measurements, *in vitro* measurements or *in silico* predictions. All parameters except those related to excretion can be estimated *in vitro*. PBTK model parameterization quality depends on the amount and quality of data available and the parameterization process can be time-consuming. Three types of parameterization, based on *in silico* methods, and optionally *in vitro* or *in vivo* data, were tested to study how the parameter values could affect the predicted internal concentrations. Two types of parameterizations (A and B) are obtained without any calibration or adjustments to fit *in vivo*

experimental data. This can be regarded as *ab initio* parameterization, although some parameters obtained in the literature can have been estimated using *in vivo* data. First, in parameterization A, QSAR models were used to predict 11 of the substance-specific parameters. Default values were used for the remaining parameters which related to oral absorption: the intestinal absorption rate was set arbitrarily to 1 hr^{-1} as in *httk* [10,26] and the absorbed fraction was set to 1 (maximizing absorption). Parameterization B makes use of available high-throughput toxicokinetic data [10,26] and *in vitro* experiments carried out specifically in the EuroMix project. *In vitro* hepatic clearance values were obtained in primary hepatocytes, and, in cases where clearance could not be quantified, screening values obtained from the literature in high-throughput assays were used. *In vitro* plasmatic unbound fractions and blood to plasma ratios from the literature were also used. In parameterization B, oral absorption parameters are also set to default values, dermal absorption defaulted to 0 except for imazalil where the proposed value was based on the mean QSAR estimates. Another parameterization (C) is based on the calibration of certain PBTK model parameters with available *in vivo* kinetic data. The characteristics of these parameterizations are summarized in Table 2.

Table 3, Table 4, and Table 5 list the values used for flutamide, linuron, dienestrol, imazalil, thiacloprid, clothianidin, cyproconazole, triadimefon, and valproic acid for each parameterization. Parameter values in rat are reported in SI, section 2.

Table 2: Characteristics of the three different parameterizations used

		Partition coefficients	Hepatic clearance	Unbound fraction	Renal elimination	Dermal and oral absorption
Estimation methods available	*in vitro*		X	X	X	
	in vivo	X	only with model calibration	X	only with model calibration	only with model calibration
Methods used	**A**	QSAR	QSAR	QSAR	QSAR	default
	B	QSAR	new *in vitro* data or literature	literature	QSAR	default
	C	QSAR or literature or calibrated *in vivo*	new in vitro data or literature or calibrated *in vivo*	literature or default 1	calibrated or default 0	default or calibrated

A- QSAR models

Partition coefficients between blood and tissues and between air and blood, unbound fraction in blood, dermal absorption rate, and renal elimination rate were estimated with the QSAR models used in the Induschem tool [27], based on the (log) octanol:water partition coefficient, volatility, and tissue composition. Hepatic clearance was estimated by using the whole-body primary biotransformation rate constant (kM) model for fish, as implemented in the BCFBAF QSAR model by the USEPA. This model is based on an evaluated database kM estimates in fish for ~700 chemicals with satisfactory predictive performance [28–30]. The allometric scaling from fish (10 gr, 15°C) to rat (200 gr, 37°C) or humans (70 kg, 37°C) and the resulting estimate of rat and human hepatic clearance has not been validated

Substance-specific parameters obtained in humans in parameterization A are reported in Table 3.

Table 3: Substance-specific parameters in parameterization A in humans

		logPC									
Substance	PCAir	Fat	Liver	Poor	Rich	Skin	Skin_sc	fub	Ke	CLH	Kp_sc_vs
Flutamide	1.96E+09	2.127	0.822	0.632	0.072	0.632	-0.699	0.06	4.29E-03	1.03	0.009

Linuron	1.03E+08	2.116	0.794	0.609	0.093	0.609	-0.699	0.082	5.92E-03	0.749	0.011
Dienestrol	8.35E+12	2.155	0.922	0.723	-0.538	0.723	-0.699	5E-04	3.87E-05	4.21	0.366
Imazalil	4.96E+08	2.144	0.874	0.680	-0.018	0.680	-0.699	0.024	1.69E-03	0.0616	0.003
Thiacloprid	1.93E+09	1.927	0.507	0.362	0.124	0.362	-0.699	0.399	2.87E-02	0.360	0.002
Clothianidin	4.83E+14	0.481	-0.201	-0.092	-0.066	-0.092	0.551	0.98	1.85E-01	0.496	3E-05
Cyproconazole	7.68E+08	2.081	0.720	0.543	0.124	0.543	-0.699	0.152	1.09E-02	0.015	0.003
Triadimefon	6.40E+09	2.058	0.679	0.508	0.130	0.508	-0.699	0.194	1.40E-02	0.0127	0.003
VPA	1.47E+05	0.497	-0.194	-0.092	-0.066	-0.092	-0.699	0.979	1.70E-01	0.0130	0.012

B- In vitro *parameters measured in substance specific experiments*

In vitro experiments were performed in order to refine the parameterization of the PBTK model by better quantifying metabolism (see SI, section 3.1 for details). *In vitro* measurements of metabolism in primary cultures of human and rat hepatocytes were obtained for dienestrol (intrinsic clearance, Table 5 in SI), linuron, and imazalil (Vmax, Km, and unbound fraction, Table 6, 7, and 8 in SI). The results are summarized in Table 4.

Flutamide metabolism parameters were refined with specific values obtained by [31–34] using *in vitro* hepatic cell line studies by in either rat cells, or human cells, or both. Flutamide is metabolized by three pathways, hydroxylation and hydrolysis into two different metabolites, all three pathways described by Michaelis-Menten saturable kinetics. At concentration levels below 1µM, the main metabolic pathway is hydroxylation (see section 3.2 in SI). Clearance was therefore estimated as the hydroxylation Vmax: Km ratio, assuming that the internal concentrations were sufficiently low compared to the Km for the kinetics to be approximated as first-order (see summary on *in vitro* data in section 3.3 of SI).

Cyproconazole, thiacloprid, and clothianidin were also tested but no kinetic constants could be determined from the *in vitro* experiments. The clearance values used for the remaining substances were therefore obtained from *in vitro* screening tests reported in the literature, in

[35] (cyproconazole, thiacloprid, clothianidin, and also triadimefon) and in [36] (valproic acid).

Unbound fractions in plasma were based on the screening *in vitro* tests reported in [37] (flutamide), by TNO and reported in R package *httk* [26] (valproic acid), and in [35] (other substances).

Substance-specific parameters obtained in humans in parameterization B with screening or more specific *in vitro* tests are reported in Table 4. Other parameters have the same values as in parameterization A

Table 4: Substance-specific parameters in parameterization B for humans obtained with screening or more specific *in vitro* tests. Other parameters have the same values as in parameterization A.

Substance	RBP [26]	fub [26]	CLH (L/hr) [26]	in vitro CLH[a] (specific test)	Kp_sc_vs (dm/hr)
Flutamide	1.20	0.0376	(531, not used)	405	0
Linuron	1.93	0.11	(545, not used)	580	0
Dienestrol	1.00	1	(0, not used)	157	0
Imazalil	2.18	0.03	(0, not used)	310	0.01
Thiacloprid	1.23	0.29	34.8		0
Clothianidin	0.745	0.51	132		0
Cyproconazole	1.24	0.11	22.9	-	0
Triadimefon	7.24	0.11	369		0
Valproic acid	0.66	0.366	2.15E-05		0

[a] Obtained with the Vmax/Km ratio or the intrinsic clearance (see SI, section 3.2)

C- Parameters calibrated using in vivo *data*

In parameterization C, in the cases of flutamide, linuron, dienestrol, thiacloprid and clothianidin, the PBTK model structure was first adapted to each substance, using existing

knowledge on kinetics from the literature. For triadimefon/triadimenol, an existing PBTK model [38] was used without further adjustment. For imazalil and valproic acid, the generic model was used for calibration.

In a second step, the following PBTK model parameters were calibrated to fit *in vivo* data for flutamide, valproic acid, imazalil, thiacloprid, and clothianidin: hepatic clearance (valproic acid, imazalil in humans), skin diffusion coefficient, fraction absorbed by gut, oral absorption rate, renal excretion rate, unbound fraction in blood (imazalil in humans), renal excretion rate (flutamide), and renal excretion rate as total clearance (clothianidin and thiacloprid). No calibration was performed for cyproconazole, linuron, or dienestrol. Partition coefficients for flutamide, thiacloprid and clothianidin were estimated using the Area Under the Curve (AUC) of *in vivo* data. Those for linuron and dienestrol were estimated by QSAR methods. The remaining parameters were set to default values or additional values retrieved from the literature, see the summary in Table 5 and section 4 in SI for details (references and distributions of substance-specific parameter values in the original PBTK models which were adapted to each case).

Table 5: Substance-specific parameters used in parameterization C in human. PCAir was set to 1e+99

	logPC												
Substance	Fat	Liver	Poor	Rich	Skin	Skin_sc	fub	Ke	CLH	Kp_sc_vs	Frac	kGut	RBP
Flutamide	0.444	0.746	0.746	0.420	0.746	0.746	0.09	1.25	405	0	0.5	0.64	1.20
Linuron	1.91	0.65	0.65	0.43	0.65	0.65	1	7.62	57.5	0	1	1	1.93
Dienestrol	2.27	1.00	1.00	0.79	1.00	1.00	0.6	9.48	283	0	1	1	1.00
Imazalil	3.6	0.7	-0.44	0.7	-0.44	-4.04	0.05	17	70	1.00E-05	0.8	10	2.18
Thiacloprid	0.0899	0.415	0.415	0.223	0.415	0.415	1	2.27	0	0	1	1	1.23
Clothianidin	-0.409	0.250	0.250	0.255	0.250	0.250	1	2.27	0	0	1	1	0.745
Cyproconazole	1.96	-0.92	-1.61	-0.92	-1.61	-3.57	0.07	0	23.4	0.1	0.996	1	1.24
Triadimefon	2.06	1.09	0.65	0.65	0.65	0.65	1	9.21	0	0	1	1	7.24

Valproic acid	-1.83	-3.1	-3.22	-2.85	-3.22	-1.39	0.14	0	4	0.1	0.996	2.88	0.66

2.1.4 Model implementation in MCRA

The MCRA system for human health chemical risk assessment, version 9.0, is available at https://mcra.rivm.nl. MCRA is a modular system for exposure, hazard and risk assessment, where doses of multiple chemicals can be modelled at either the external level (specifying an exposure route) or the internal level (specifying a target organ). MCRA contains a module *Kinetic models*, where various PBTK models have been linked in, such as the EuroMix generic PBTK model described in this paper. The EuroMix PBTK model can be run with any parameter set: both human and animal models can be specified. Alternatively, if no kinetic model is available, simple absorption factors can be specified to characterize the ratio between internal and external doses. Given a PBTK model, several parameterizations of it can be defined for different substances and biological systems (e.g. rat, human). The parameter values for each instance of a model are specified in data files that have to be uploaded to the MCRA platform. For the nine substances in this paper, the parameter data files are available on the EuroMix data share. In MCRA runs with kinetic models, the user specifies the internal compartment that is of interest and the time period that the PBTK model should be run. Internal doses for chronic risk are quantified by the average internal dose as calculated by the PBTK model past a user-specified start time point (to avoid the build-up phase of a pseudo-steady state). Internal doses for acute risk are quantified by the average internal peak level in this same period. For exposure assessments, daily exposure values as inputs are derived from the dietary exposure module of MCRA or from externally provided exposure values for non-dietary exposure [12,39]. For hazard characterizations, the input dose corresponds to e.g. the

acceptable daily intake (ADI) or acute reference dose (ARfD), which is then provided as an input to the PBTK model once per day.

The EuroMix generic PBTK model, version 6 (see https://doi.org/10.5281/zenodo.3553689) was implemented as a set of ordinary differential equations (ODE) written in C and compiled into a dynamically linked library (DLL). The R package deSolve [40,41] is used to load the DLL into R. Compiled code has the benefit of increased simulation speed. This may be a significant gain especially when the model is applied multiple times which is current practice in MCRA. Through the use of a forcing function time series, external input variables (*i.e*. the administered doses) are fed into the model and the solver will interpolate between the different timesteps. (Example code written in R is available on request).

2.1.5 Model calculations

Calculations were performed with MCRA 9.0, R version 3.6.1 [20], packages httk [26], deSolve [40], EnvStats [42], sensitivity [43].

2.2 Model verification

The internal concentrations predicted by the PBTK model were compared to *in vivo* data on kinetics for each of the 9 chemicals. Area Under the Curve (AUC) and maximal concentration (Cmax) of kinetics were also computed and compared to *in vivo* data. *In vivo* data on kinetics was collected from the literature in humans and rats (Table 6 hereafter, Table 23 in section 6.1 in SI). Human *in vivo* data on internal kinetics was only available for flutamide, valproic acid and imazalil in blood, plasma or urine.

With parameterization C, where some parameters specific to flutamide, valproic acid, imazalil, thiacloprid, or clothianidin were calibrated to fit *in vivo* data, prediction quality is assessed using data that served for calibration and additional data, used as validation datasets

[44–51], when available. For imazalil, thiacloprid and clothianidin, no additional data was available: quality of prediction could not be assessed on data which had not been used to calibrate the model. Quality of prediction for valproic acid and flutamide was assessed on all data together and also on the additional data.

Table 6: Summary of available *in vivo* data on kinetics. Details are provided in supplementary information, section 6.1

Substance	Number of studies		Organ / Tissue	Dosing	Dose	Duration (hours)	Calibration data	Validation data
	Human	Rat						
Flutamide	2	1	plasma	Oral, single (2), multiple (1)	250, 500, 750 mg/day (human) 15 mg/kg (rat)l	72	[52]	[51]
								[44]
Valproic acid	6	1	Serum (3), plasma (5), blood (1)	Oral (6), IV (6) Single (8), twice (2)	250-1000 mg or 15 mg/kg (human) 10-600 mg (rat)	110	[53]	[45], [46], [47], [48], [49]
							[54]	[50]
Triadimefon / triadimenol	-	1	plasma, liver, kidney, brain, fat	IV single	50 mg/kg	24	[38]	
Imazalil	1	-	Urine (2)	Oral (1), Dermal (1)	0.025 – 0.05 mg/kg	120	unpublished data (Faniband et al.)	
Thiacloprid*	-	1	Organs, plasma, excreta	Oral (4), IV (1) Single (4)	1 - 100 mg/kg	72	[55] (unpublished data)	

				multiple (1)				
Clothianidin*	-	2	organs, plasma, excreta, blood	Oral Single (6), multiple (1)	2.5 - 250 mg/kg		168	[56] (unpublished data), [57]
Cyproconazole*	-	1	Organs, excreta	Oral (3) IV (1)	0.5 - 130 mg/kg		168	[56] (unpublished data)
Linuron	-	-					--	
Dienestrol	-	-					--	

*: Only measured as total radioactivity, not parent substance.

Data on flutamide and valproic acid were collected in both species. For imazalil, as data was only found in humans, only the human parameterization of the PBTK model was checked. Conversely, for thiacloprid, clothianidin, cyproconazole, and triadimefon, data was only available in rat, and so only the rat parameterization was checked. The data collected in rats on thiacloprid, clothianidin, and cyproconazole was expressed as equivalents of administered dose and not as levels of the parent substance, as it was obtained using autoradiography. Metabolism was the main elimination pathway for thiacloprid and cyproconazole [55,56] and occurs to a lower extent for clothianidin [56]. Body weight was set to the value reported in the studies if specified, or with a default 57 kg for women and 75 kg for male if gender was specified, or to a default 70 kg.

Levels were often quantified in plasma rather than blood, therefore blood concentrations predicted by the PBTK model were converted to plasmatic concentrations when necessary, using a default value of 1 in parameterization A and the substance-specific blood to plasma

ratio (RBP) [26] in parameterizations B and C (see Table 4 and Table 5 for humans, and section 2 of SI for rats).

2.3 Using the PBTK model in cumulative risk assessment

In the absence of PBTK models or other kinetic information, the default assumption in MCRA is that the internal concentration at the target organ is equivalent to the daily exposure per unit of bodyweight. This is defined as a kinetic absorption factor equal to 1. For chronic risk assessments, the kinetic absorption factor is defined as the ratio of the mean concentration at target organ divided by the mean exposure per unit of bodyweight and per day. For acute risk assessments, the kinetic absorption factor is defined as the ratio of the maximum calculated concentration at target organ within a day (averaged over multiple days) divided by the mean exposure per unit of bodyweight and per day.

The assumption of a kinetic absorption factor equal to 1 is met in a variety of situations, for example, with oral exposure, if the chemical is totally absorbed, uniformly distributed in the body, and that, in acute exposures, it is not eliminated, or that in chronic risk assessment, it does not accumulate. A variety of other scenarios could be envisaged that result in a kinetic absorption factor equal to 1, for example when the absorbed fraction is lower but the substance bioconcentrates at the target organ, or else when the substance has a high affinity for the target organ but its elimination compensates for that higher affinity. The availability of a kinetic model allows risk assessors to refine the default assumption on the kinetic absorption factor by using the predicted internal concentrations.

The Euromix generic PBTK model implemented in MCRA includes one term for non-linearity, the Michaelis-Menten metabolism kinetics. In this application to nine substances, metabolism was modelled as first-order kinetics (clearance) and therefore the model is linear: the internal concentrations are proportional to the external dose at steady state. The kinetic

absorption factors and relative concentration factors between substances can therefore be used whatever the dose levels investigated, both for interspecies extrapolation and for inter-chemical relative potency factors (RPFs).

Once the model performance with the various parameterizations had been checked against *in vivo* data, the impact of the various parameterizations on critical steps of cumulative risk assessment was evaluated. Internal concentrations in a 70 kg human at steady state were predicted under continuous exposure scenarios with 1 mg/kg BW/day of each substance. A continuous exposure scenario was used rather than daily repeated exposure in order to avoid sharp peaks in concentration levels. Steady state was defined as being achieved when the concentration at a given time was greater than 95% of the concentration reached at twice this time. When steady state was not achieved, the simulations were stopped at 2000 days in humans. The predicted internal concentrations were analyzed in the following ways:

1- The human blood concentrations were compared to those obtained in rat for use in interspecies extrapolation.
2- Kinetic absorption factors at steady state were also calculated. These factors allow extrapolation of internal to external dose-response relationships and can be used to compare kinetics inside each cumulative assessment group.

It is important for risk assessment to take into account both uncertainty in model parameter values and variability of those parameters in the population. In this work population variability on physiological parameters was ignored but uncertainty on substance-specific parameters was quantified. Commonly assumed uncertainty factors on partition coefficients, transport rates and metabolic parameters are 3-fold factors (e.g. [15-19]). When the PBTK model was used to predict internal concentrations, 10,000 Monte Carlo simulations were performed in each exposure scenario, by simulating uncertainty on partition coefficients

(whilst maintaining proportionality amongst them), oral absorption rate, fraction absorbed, hepatic clearance, elimination rate and unbound fraction in plasma. For more details, see section 5.1 in SI. Furthermore, when uncertainty around substance-specific parameter values was not reported in the sources, the uncertainty was modelled with the same distribution whether the value was obtained from the literature or was a default value and is reported in SI, section 5.2.

3 RESULTS

3.1 Model evaluation

3.1.1 A data-rich situation: valproic acid

Predicted valproic acid (VPA) concentrations in plasma were compared to the data collected from the literature (Figure 2 and Figure 3). The PBTK model for VPA had been calibrated on data in obtained in rats by [54] and [32]. The substance-specific parameters for valproic acid are reported in Table 7. With parameterizations A and B, although most predictions were within a 10-fold factor of the observations, the kinetics did not match observed kinetics since barely no decrease was observed during the study duration. Parameterization C provided overall better predictions in rats and in humans (Table 8): in humans, 72% of predictions were within a 2-fold factor. When kinetics were summarized as the AUC or the Cmax (Table 9 and Table 10), Parameterization C also provided better predictions in particular in humans (where a large number of individual curves were available), but not in rats, where the concentrations were slightly over predicted. The results show that in humans both repeated and unique exposures are well described by the model especially with parameterization C (Figure 3).

With parameterizations A and B, due to low hepatic clearances, VPA is predicted to accumulate in the body, which is not observed *in vivo*.

Table 7: Rat and human PBTK parameters for valproic acid.

			logPC					
Parameters	Species	PCAir	Fat	Liver	Poor	Rich	Skin	Skin_sc
A	Human	1.47E+05	0.497	-0.194	-0.092	-0.066	-0.092	-0.699
B	Human	1E+99						
C	Human	1E+99	-1.83	-3.1	-3.22	-2.85	-3.22	-1.39
A	Rat	6.53E+04	0.057	-0.036	-0.201	-0.027	-0.201	-0.699
B	Rat	1E+99						
C	Rat	1E+99	-1.83	-3.1	-3.22	-2.85	-3.22	-1.39

Parameters	Species	fub	Ke	CLH	Kp_sc_vs	Frac	kGut	RBP
A	Human	0.979	0.170	1.30E-02	0.012	1	1	1
B	Human	0.366	7.5	2.15E-05	0	1	1	0.66
C	Human	0.14	0	8.8	0.1	0.996	2.88	0.66
A	Rat	0.979	0.00186	2.35E-04	0.035	1	1	1
B	Rat	0.22	0.0786	1.09E-07	0	1	1	0.600
C	Rat	0.14	0	0.4	0.1	0.996	0.3	0.600

Parameterization A and B are akin to a worst-case scenario regarding absorption. In the case of acute exposure scenarios, though, the maximal concentration is underestimated, which can be problematic in a risk assessment context. Over-predictions in this deterministic solution (nominal run) may be due to high levels of absorption. When uncertainty is added, the absorbed fraction will often be lower which may result in more accurate predictions. Furthermore, it must be noted that even in data-rich situations such as valproic acid, when a substance-specific PBTK model is translated into the generic PBTK model, results can be more uncertain than with the original model.

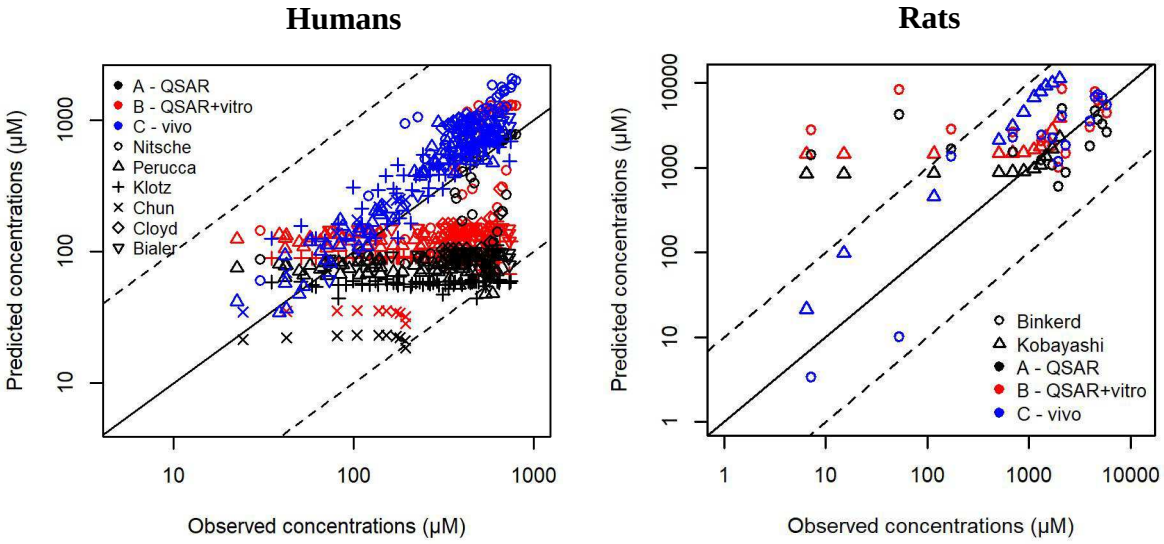

Figure 2: Predicted vs. observed plasmatic, serum or blood valproic acid (VPA) concentrations in humans or rats. Symbols refer to the source studies. Black= parameterization A (QSAR), red= parameterization B (QSAR+vitro), blue = parameterization C (vivo). Dotted lines denote 10-fold deviations.

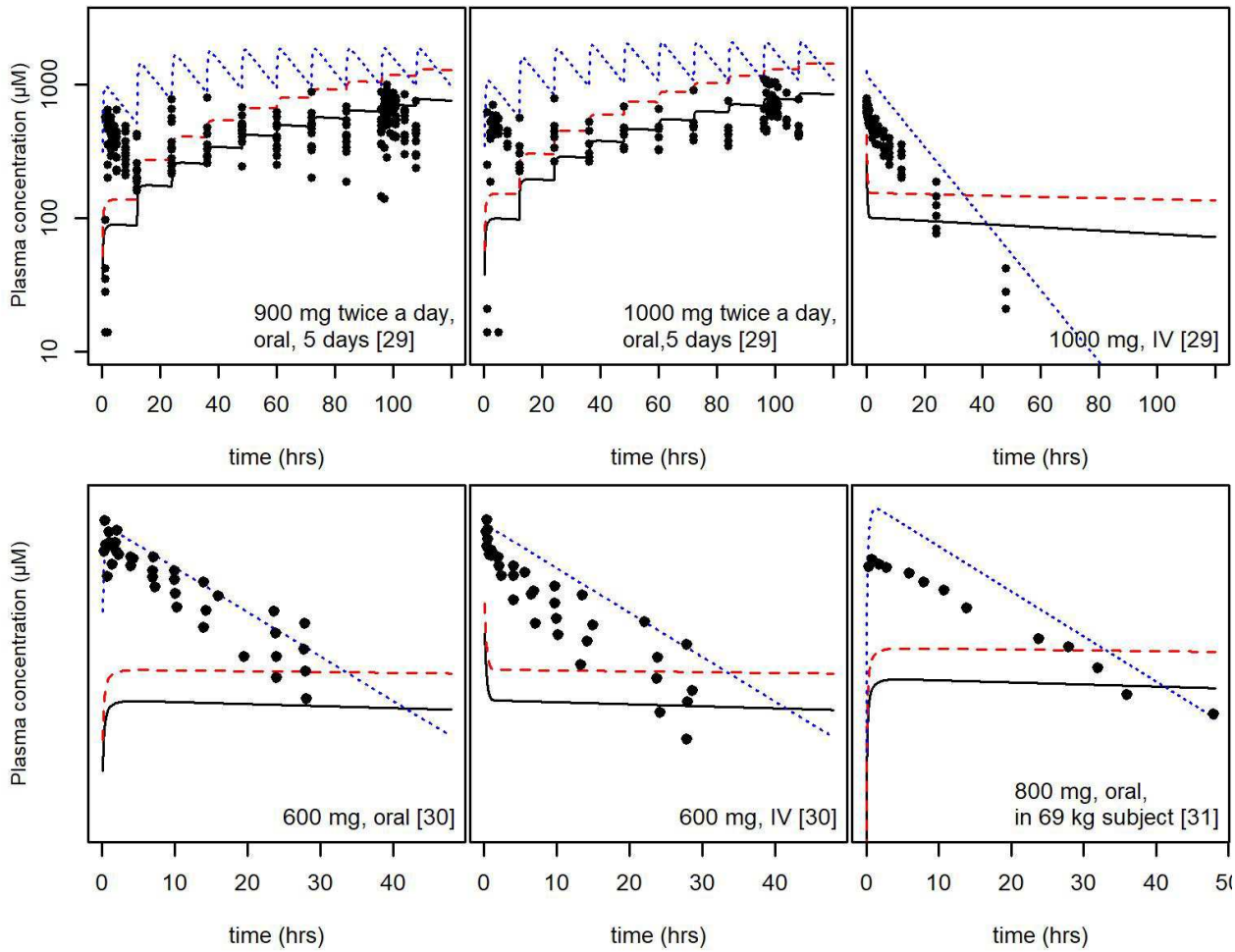

Figure 3: Predicted and observed plasma VPA concentrations in humans, according to parameterizations A (plain curve), B (dashed curve), and C (dotted curve). In [48], serum rather than plasma concentrations were reported.

Table 8: Percentage of VPA concentration predictions within a 10- and 2-fold factor of the observations

Species	Parameterization		
	A	B	C
Human (10-fold difference)	96.7	99.6	100.0
Human (2-fold difference)	28.4	32.5	72.0
Rat (10-fold difference)	84.0	76.0	100

Rat (2-fold difference)	52.0	60.0	40.0

Table 9: Percentage of VPA predicted AUC within a 10- and 2-fold factor of the observed AUC

Species		Parameterization		
		A	B	C
Human (20 simulations)	10-fold difference	100	100	100
	2-fold difference	15	55	75
Rat (3 simulations)	10-fold difference	100	67	100
	2-fold difference	0	33	33

Table 10: Percentage of VPA predicted Cmax within a 10- and 2-fold factor of the observed Cmax

Species		Parameterization		
		A	B	C
Human (20 simulations)	10-fold difference	85	100	100
	2-fold difference	10	20	85
Rat (3 simulations)	10-fold difference	100	100	100
	2-fold difference	100	100	67

3.1.2 Effect of parameterizations on the predictive performance of the generic PBTK model

The PBTK model predictions for the nine selected chemicals, using the three parameterizations, were compared to the *in vivo* data on kinetics collected from the literature (Table 6), obtained with oral or intravenous administration (section 6.2 in SI for humans and section 6.3 in SI for rats). Concentrations in blood or plasma, liver, skin, and fat were predicted with the model; summaries of the comparisons with observations are provided in Figure 4, Figure 5, and Table 11. With thiacloprid, clothianidin, and cyproconazole in rats, data represented both parent substance and metabolites; concentrations are therefore expected to be underpredicted. For these substances, graphical comparisons of predictions and observations are provided (section 6.4 in SI) but the deviation factors are not reported since they can be misleading.

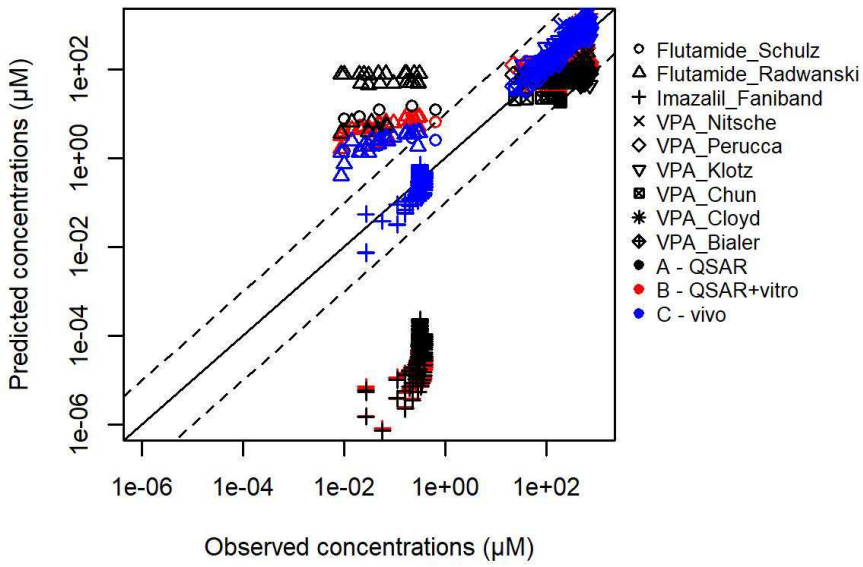

Figure 4: Predicted vs. observed plasmatic concentrations (flutamide and VPA) or cumulated amounts excreted in urine (Imazalil, µmol) in humans. Black = parameterization A (QSAR), red = parameterization B (QSAR+vitro), blue = parameterization C (vivo).

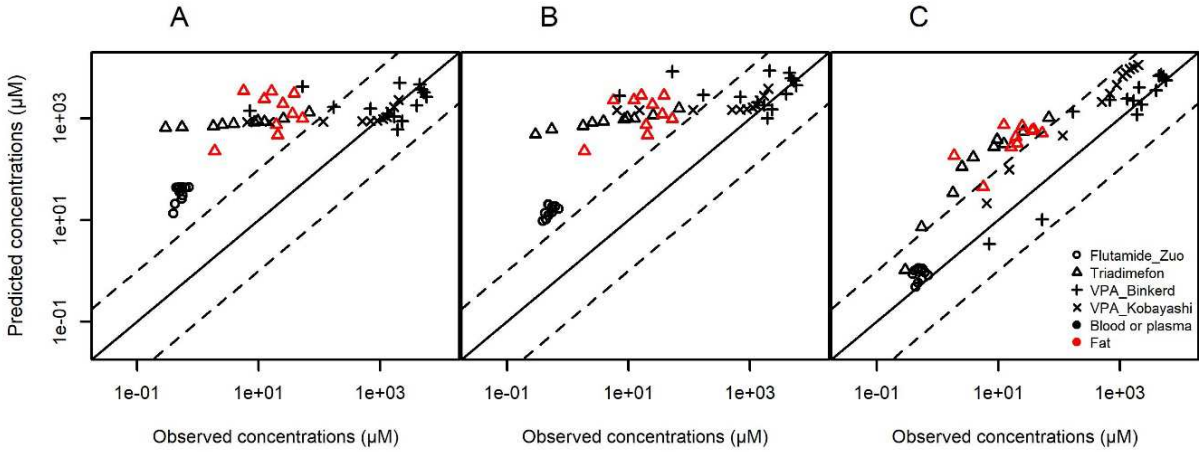

Figure 5: Predicted vs. observed concentrations in rats for flutamide, triadimefon, and VPA with parameterizations A, B, and C.

Table 11: Percentage of predictions within a 10-fold factor of the observations

	Species	Parameterization		
		A	B	C
Flutamide	Human	0	0	6.25
Imazalil	Human	0	0	100
Valproic acid	Human	96.7	99.6	100
Flutamide	Rat	0	0	100
Thiacloprid	Rat	64.3	86.7	70.4
Clothianidin	Rat	63.8	33.0	55.3
Cyproconazole	Rat	17.9	21.4	14.3
Triadimefon	Rat	0	0	15
Valproic acid	Rat	84	76	100

Quality of predictions was assessed by computing fold deviations from observations over the limit of quantification (Table 11). With the human data and parameterization A or B, overall, 56 and 58% of the predictions were within a 10-fold factor; in both parameterizations, predictions for flutamide, imazalil, and triadimefon where never within a 10-fold factor. With parameterization C where some model parameters were calibrated, overall 93% of the predictions were within a factor 10 and for all substances. In some cases, small changes in parameterization provided significant improvements. For instance, predictions of the amount of imazalil excreted in urine were improved by increasing the urinary elimination clearance.

With parameterization C, in the cases where validation datasets where available, i.e. for valproic acid in humans, and for flutamide and valproic acid in rats, all predictions were within a 10-fold interval, including the validation datasets, which were generally less well predicted in parameterizations A and B. For flutamide in humans, with parameterization C, predictions quality was similar in both validation and calibration datasets: 2 out of 5 data points in the validation dataset were within a 10-fold interval compared to 6.25% overall.

With the rat data - excluding radioactivity data – parameterization A and B provided overall similar but not very good predictions: respectively 37% and 34% of the predictions were within a 10-fold factor. With parameterization C, 70% were within a 10-fold factor. The time-course of plasmatic levels (including the AUC and the Cmax) is better predicted in parameterization C.

According to Figure 4 and Figure 5, flutamide concentrations in the dataset from Radwanski et al. [52] (humans) and in Zuo et al. [44] (rats) were better predicted with parameterization B than with parameterization A due to *in vitro* hepatic clearances which were over 2 orders of magnitude higher than the QSAR estimates. A sensitivity analysis of blood concentrations after repeated ingestion of flutamide every 8 hours confirmed the importance of hepatic

clearance, by showing that with parameterization B, hepatic clearance, unbound fraction in blood and, to a lesser extent, absorbed fraction were by far the most influent parameters (see SI section 8). Predicted triadimefon concentrations in rats with parameterization B were not significantly improved compared to parameterization A, although the *in vitro* hepatic clearance was also higher than the QSAR estimate. Even with *in vitro* clearance estimates, the internal concentrations were overestimated for both substances.

3.2 Comparison of concentrations in blood between species and between substances

The impact of the parameterization on relative concentration factors between human and rat, and between substances was evaluated using continuous exposure scenarios. Predictions for continuous 100-day oral administration of 1 mg/kg/day of each substance are shown in section 7 in supplementary information.

Steady-state, here defined as 95% of the maximum predicted value, was not always achieved after 100 days of continuous exposure (see Table 12 and Figures 1 to 6 in Section 7 in SI). Parameterization A does not achieve steady-state even at 2000 days (over five years) for dienestrol, imazalil, cyproconazole, and triadimefon.

Table 12: Substances for which steady state is not achieved after 100 days or 2000 days continuous exposure in humans

	100 days	2000 days
A QSAR only	flutamide, linuron, dienestrol, imazalil, thiacloprid, cyproconazole, triadimefon	dienestrol, imazalil, cyproconazole, triadimefon
B QSAR + in vitro	valproic acid	-
C in vivo	-	-

In particular in humans, with increasing parameterization complexity, the predictions varied more widely from one substance to another (Figure 6). In particular, with parameterization A, elimination and metabolism were relatively small for all substances. Predictions in human at

2000 days were generally higher than in rat at 100 days (except for linuron in parameterization B and C and Triadimefon in parameterization B), although in humans in parameterization A, some substance concentrations had not reached steady-state.

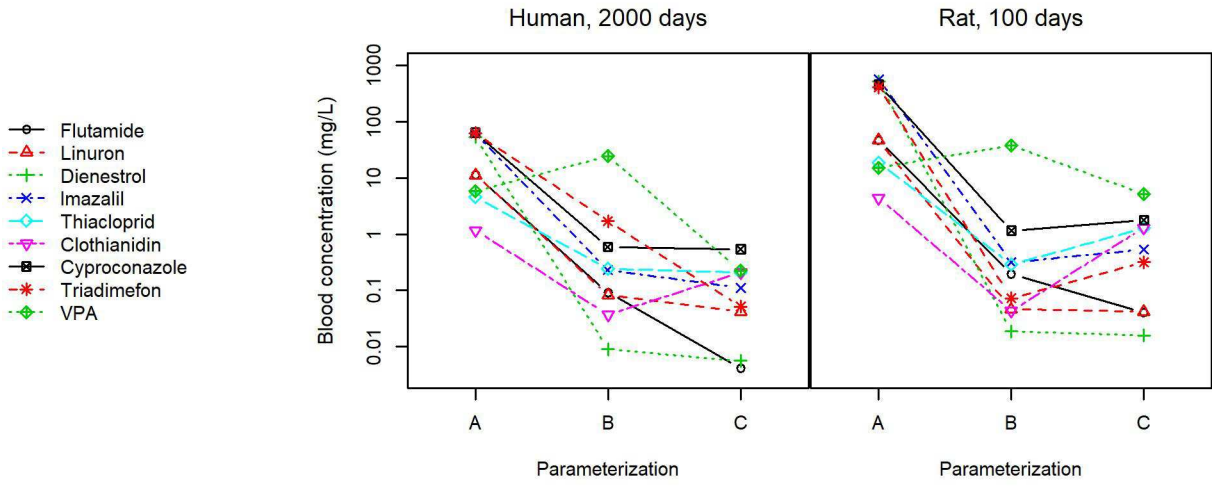

Figure 6: Predicted steady-state arterial concentration after exposure to 1mg/d/kg in humans and rats for each parameterization

The concentrations predicted at steady-state with parameterization B were generally lower than those obtained with parameterization A, due to faster metabolism in the former. The *in vitro* hepatic clearances were indeed larger than those estimated with QSAR models except for valproic acid. With parameterization C, predicted internal concentrations were lower still in particular for flutamide. The various *in vitro* tests were mostly in agreement with each other: the clearance values obtained were within a factor 2 of the values obtained from the literature for linuron [35] and flutamide [37]. For imazalil in rats, the value was 4-fold lower than the value from the literature [35]. Using a clearance value rather than Michaelis Menten kinetics appears to be an acceptable approximation for linuron and imazalil: hepatic concentrations predicted in the various exposure scenarios were indeed lower than the value of Km.

The uncertainty around the predicted relative concentrations in human blood following a chronic 2000 day-exposure of the nine chemicals belonging to three cumulative assessment groups is represented in Figure 7, according to the three different parameterizations. In both humans and rats, the parameterization had most impact on predicted flutamide and dienestrol levels in blood; it had the least impact on thiacloprid and valproic acid. No relationship could be drawn between the amount of data available and the extent to which parameterization affected predictions. In all cumulative assessment groups, the overall level of arterial concentrations varied, as well as the concentrations of substances relative to each other (Figure 7).

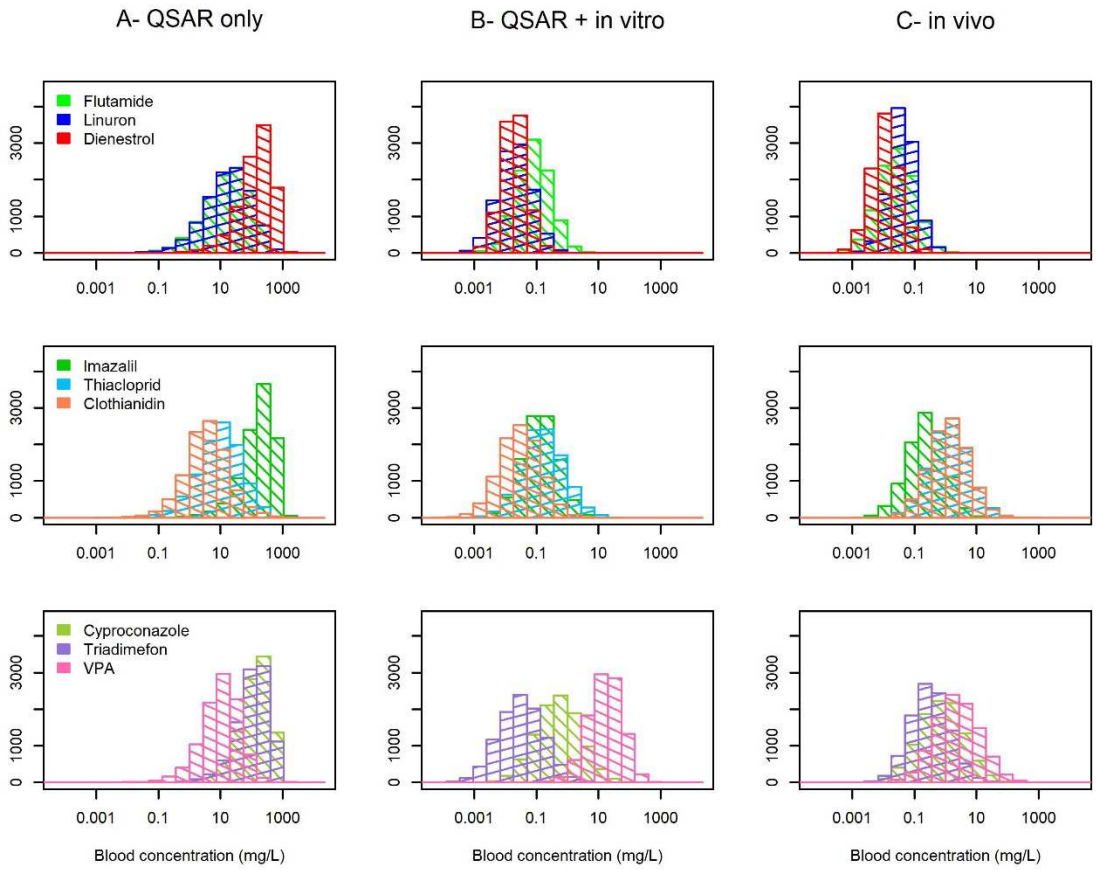

Figure 7: Predicted arterial blood concentrations after 2000 days continuous exposure to 1mg/kg/day in humans in each cumulative assessment group using parameterizations A, B and C.

3.3 Kinetic absorption factors

The kinetic absorption factor values represented in Figure 8 were obtained using liver concentrations over 100 days of continuous exposure. With parameterization A, they are mainly greater than 1. With parameterization B and C, they are mainly smaller than 1 in the flutamide-linuron-dienestrol group, and variable for the two other groups.

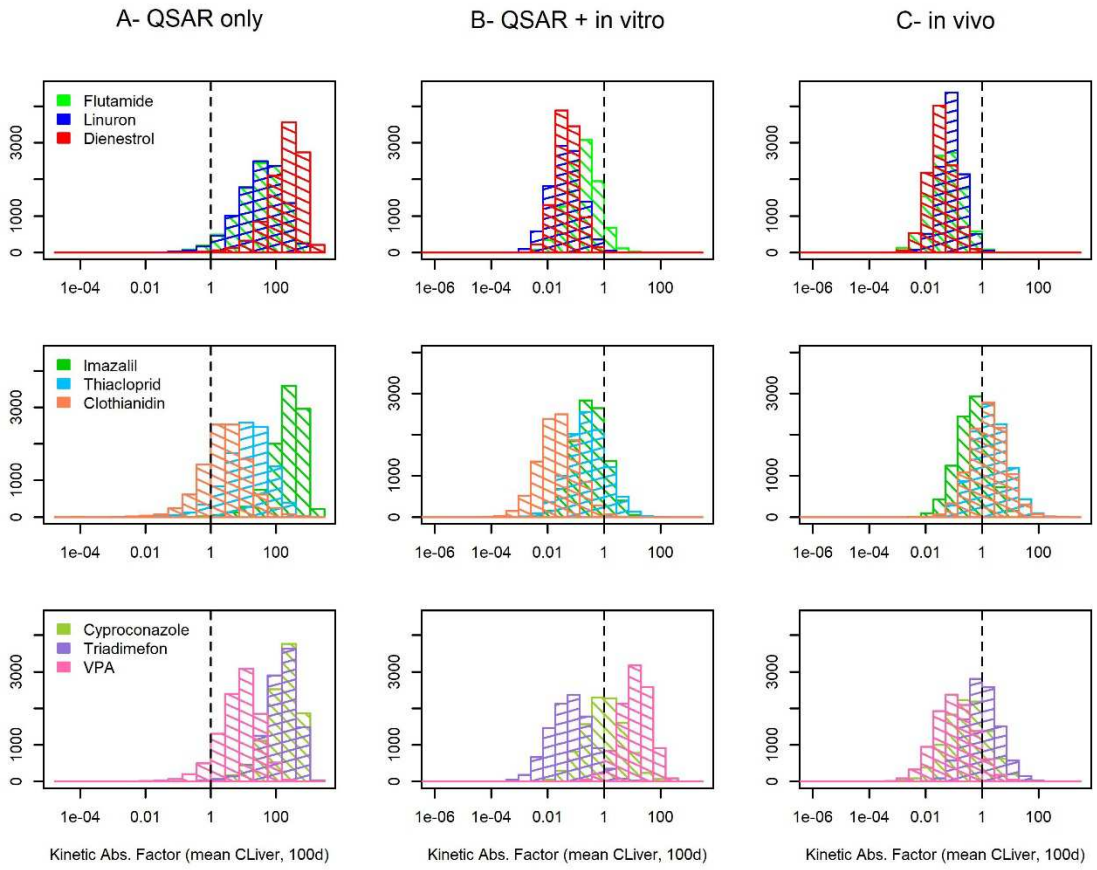

Figure 8: Kinetic absorption factors in humans resulting from a 2000-day continuous exposure in each cumulative assessment group using parameterizations A, B and C, calculated as a ratio of the mean hepatic concentration over 100 days over the daily exposure per unit bodyweight. A value of 1 implies the hepatic concentration equilibrates with the daily dose per unit body weight.

4 DISCUSSION

In the present paper, various *ab initio* parameterizations of the EuroMix generic PBTK model included in the MCRA platform were tested on nine substances belonging to three cumulative assessment groups. This paper illustrates how the level of knowledge used in the parametrization of PBPK models can affect their predictive capabilities. We also showed the impact on the parametrization on model outputs used in chemical risk assessment. In the current implementation in MCRA, the PBTK model for humans translates an external human exposure to an internal exposure and vice versa. The kinetic model includes three major exposure routes in order to model dermal, oral, and inhalation exposures and can, therefore, be applied to a variety of chemicals and exposure scenarios. The model can also be used to determine to which extent each exposure route contributes to the kinetics of a single chemical in each compartment and model kinetics of binary mixtures with metabolic interactions. In the hazard characterization part in MCRA, the first points of departure from animal studies are translated to a human internal concentration equivalent using an interspecies factor rather than the animal PBTK model.

Detailed modelling requires detailed data which is not always available. Intestinal absorption rates, fraction absorbed, and elimination rates, either specifically as hepatic clearances, or more generally as elimination from blood, were often missing. As *in silico* or *in vitro* estimates of absorption were not available, default values were often used for the two intestinal absorption parameters although there is evidence that, in particular in non-pharmaceutical substances, the observed absorbed fraction may be smaller than our default value of 1 and that intestinal absorption rate can vary significantly between substances [58]. The estimate of metabolic clearance using ab initio (QSAR) predictions is notably uncertain

as it involves multiple assumptions, that a) fish whole body clearance is mainly due to hepatic clearance, b) the fish hepatic clearance is an indication of (similar) rat and/or human hepatic metabolization, and c) that this can even be extrapolated quantitatively (applying allometric scaling). One obvious way to improve on the generic QSAR model estimations is to use a similar model specific for human whole body clearance [59] which has only recently become publicly available in a software implementation (QSARINS package, http://www.qsar.it/). Metabolic clearances were in some cases underestimated when based on in vitro data, either due to limitations of the in vitro assays or because metabolism may occur at sites other than the liver. Metabolic clearances were in some cases underestimated when based on *in vitro* data, either due to limitations of the *in vitro* assays or because metabolism may occur at sites other than the liver.

The quality of prediction with the various parameterizations was variable. In parameterization C, a small number of parameters (most often: unbound fraction, renal excretion rate, absorbed fraction and intestinal absorption rate, depending on the substance) were calibrated to fit *in vivo* data for flutamide, clothianidin, imazalil and valproic acid. The results with *in vivo* parametrization were, as expected, in better agreement with the data than the *ab initio* parameterizations. In the data-rich case of valproic acid, most predictions were within a 2-fold factor of the observations, which is generally considered adequate [2]. Estimates of oral absorption and total clearance parameters were very uncertain and could be improved if input from *in vivo* data was available.

Use of worst-case default values for absorbed fraction (1) and metabolism and elimination may lead to overestimation of internal concentrations, in particular under chronic exposure scenarios. The worst-case intestinal absorption rate could depend on each chemicals' toxicokinetic and toxicodynamic characteristics and on the type of exposure scenario (acute or

chronic): high intestinal absorption rates result in short duration peak concentrations if the chemical is rapidly eliminated. In this case, uncertainty and variability on the absorption rate translate into uncertainty on the peak internal concentrations which are used in acute exposure risk assessment. On the other hand, low values of absorption rate are unrealistic in this PBTK model since they would lead to accumulation in gut. With our PBTK model, a value of 1 hr^{-1} appears to be already relatively high, since sensitivity analysis showed that blood concentrations after repeated exposure to flutamide in parameterization B (*kGut*=1 hr^{-1}) were not sensitive to variations in intestinal absorption rate. The default value for absorption rate in the *httk* package has been changed in more recent versions from 1 hr^{-1} to an even higher value of 2.18 hr^{-1}. With our model, when using default values for metabolism and elimination, inaccuracies in predictions are likely to occur under chronic exposure scenarios with chemicals which are predicted to accumulate, due to low metabolism or poor elimination and accumulation of prediction errors over time. Furthermore, default values carry a large amount of uncertainty. When PBTK model parameters are obtained with *ab initio* approaches, such as with QSAR models, rather than from default values, the uncertainty on parameter values can be quantified and propagated in the PBTK model predictions.

Overall, the use of default values illustrates how risk assessment tools must compromise between data availability and model complexity [7,60]: calibrating parameters is time-consuming, and requires large amounts of *in vivo* data for the model to be predictive in dose-to-dose extrapolation. Under the perspective of providing PBTK models for a large number of substances in lower-tier calculations, *ab initio* models that rely upon existing databases or simple QSAR models are more feasible. Their use is increasingly encouraged; many databases of physico-chemical parameters and *in vitro*-determined ADME properties are available and have been recently described [61]. Data from several sources could be combined in a meta-analysis before being used as input in PBTK parameterization. This

approach can also provide indications on the uncertainty and variability of the parameters. Recent examples of application show how the *httk* package can be used to analyses *in vitro* toxicity databases [62]. On the other hand, it is a bonus for higher-tier calculations if more complex models are also available. For cypermethrin, for example, precise simulations require diffusion-limited models [63], which is a complexification of all the flow-limited model implemented in MCRA: simpler models will imply sacrifices on precision and accuracy of the predictions. More complex kinetic models can be specified and linked to MCRA.

The PBTK model can be used to compare bioaccumulation and kinetics of substances belonging to the same cumulative assessment group. Deriving relative concentration factors required choosing the tissue of interest and the metric used to summarize the kinetics of each substance in that tissue. In the present paper, kinetics were summarized by a steady-state concentration level in the case of continuous exposures, but, in the case of single or repeated boluses, relative internal concentration factors can also be derived on the basis of other kinetic metrics. The most commonly used kinetic metrics in relation to adverse effects are peak concentration, mean daily amount, steady-state-concentration, or Area Under the Curve (AUC) of the parent compound or one of its metabolites in blood or target tissue [64,65]. Short-term effects are generally characterized by peak or mean concentration in blood or target tissue [66], whereas longer-term effects tend to be predictable by cumulative measures of dose in tissues, as in MCRA. Attention is drawn to the fact that, in the case of chemicals that accumulate under chronic exposure scenarios, overestimation of internal concentrations which can occur with the use of default values. In cumulative risk assessment, these inaccuracies can build up to substantial overestimation of the risk. Furthermore, depending on the compound, metrics can be more appropriate when estimated using unbound rather than total concentrations [67]. All these metrics can be computed in MCRA.

In the present paper, relative concentration factors between substances were estimated based on blood concentration levels, because blood is an important biomarker of exposure and because blood level predictions are the least uncertain: *in vivo* data on kinetics in humans were often only available in this compartment. Here, the model was linear, therefore relative concentration factors based on steady-state concentrations are independent of the dose levels. In turn, these factors can allow conversion of internal relative potency factors (RPFs) between substances obtained from *in vitro* data into external RPFs in humans by translating internal concentrations into external doses. An example is provided in [12]. Furthermore, internal interspecies (as opposed to within-species) relative potency factors (RPFs) can also be estimated by multiplying the external interspecies RPFs by the kinetic absorption factor.

In practice, within the context of cumulative risk assessment, realistic exposures are rarely constant, which means that internal concentrations fluctuate, and steady state is not achieved. When substances exhibit differences in internal kinetics, their internal concentration ratio will also be time-dependent, even if they are present in the environment or food at a constant ratio. In this case, the internal RPFs (within-species and between species) cannot be directly converted into an external RPF because internal concentration factors determined at steady state are no longer valid. This is of particular concern when at least one of the chemicals is absorbed and eliminated rapidly. Indeed, in case of an acute simultaneous exposure for example, if the response at the cell level is immediate, the difference in time of peak concentration may imply that once one chemical has reached the tissue, the other may no longer be present. In this case, predicting the response based on an addition of both peak concentrations could be a very conservative approach, which would overestimate the risk under the assumption of absence of any toxicodynamic interaction, and may call for a more sophisticated use of the PBTK model.

IVIVE integrates kinetic modelling into a larger goal of extrapolating the magnitude of effects. Using the internal kinetics, responses to mixtures, i.e. toxicodynamics, can be predicted, provided the dose-response relationship of each substance in a cumulative risk assessment group can be accurately modelled. However, this relies on several assumptions such as (i) dose additivity, (ii) absence of any carry-over effect [68], and (iii) relevance of the metric chosen to summarize internal kinetics towards the dose-response relationship. (i) As mentioned in the previous paragraph, dose-additivity may be a simplistic approach when steady-state is not achieved, because of the fluctuations in relative concentrations of the various compounds. (ii) Carry-over effects from one chemical to another occur when one substance has a lasting effect on the response to the other chemical, although it has been cleared from the target tissue. (iii) The metric chosen to represent the kinetics can be determinant on whether the response has time to occur. Unfortunately, data on dose-time-relationships is rarely available, and therefore summarizing the predicted time-course of the concentration at the target organ is a convenient simplification. Adverse Outcome Pathways (AOPs) often include an implicit time-scale which depends on the level (molecular, cellular, tissue, or whole-body) at which each key event occurs [69,70]. In the perspective of modeling quantitative AOPs, PBTK models can provide an internal dose to be related to the molecular initiating event.

5 CONCLUSION

The generic PBTK model developed has been implemented in the MCRA platform to be used in cumulative risk assessment. The model can convert external exposure to organ-level exposure and can quantitatively extrapolate *in vitro* exposures to *in vivo*, as in the QIVIVE module of MCRA. The PBTK model implemented is generic regarding the substance and in

many cases will not take into account some physico-chemical or biological specificities of substances. The model can however be used in data-poor situations, so that calculations can be done for large numbers of chemicals. When used as more than a screening tool, accurate predictions call for a large amount of data and time-consuming adjustments of the model to fit the data.

When associated with *in vitro* dose-response data, the PBTK model can provide either interspecies or inter-chemical potency factors at steady state, which is relevant for chronic risk assessment. Uncertainty factors can however be large, covering several orders of magnitude.

6 ACKNOWLEDGMENTS

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8 FIGURE LEGENDS

Figure 1: Schematic representation of the EuroMix generic PBTK model implemented in the MCRA platform.

Figure 2: Predicted vs. observed plasmatic, serum or blood valproic acid (VPA) concentrations in humans or rats. Symbols refer to the source studies. Black= parameterization A (QSAR), red= parameterization B (QSAR+vitro), blue = parameterization C (vivo).

Figure 3: Predicted and observed plasma VPA concentrations in humans, according to parameterizations A (plain curve), B (dashed curve), and C (dotted curve). In [48], serum rather than plasma concentrations were reported.

Figure 4: Predicted vs. observed plasmatic concentrations (flutamide and VPA) or cumulated amounts excreted in urine (Imazalil, µmol) in humans. Black = parameterization A (QSAR), red = parameterization B (QSAR+vitro), blue = parameterization C (vivo).

Figure 5: Predicted vs. observed concentrations in rats for flutamide, triadimefon, and VPA with parameterizations A, B, and C.

Figure 6: Predicted steady-state arterial concentration after exposure to 1mg/d/kg in humans and rats for each parameterization

Figure 7: Predicted arterial blood concentrations after 2000 days continuous exposure to 1mg/kg/day in humans in each cumulative assessment group using parameterizations A, B and C.

Figure 8: Kinetic absorption factors in humans resulting from a 2000-day continuous exposure in each cumulative assessment group using parameterizations A, B and C, calculated

as a ratio of the mean hepatic concentration over 100 days over the daily exposure per unit bodyweight. A value of 1 implies the hepatic concentration equilibrates with the daily dose per unit body weight.

9 TABLE LEGENDS

Table 1: Physiological parameter values used for the Euromix generic PBTK model

Table 2: Characteristics of the three different parameterizations used

Table 3: Substance-specific parameters in parameterization A in humans

Table 4: Substance-specific parameters in parameterization B for humans obtained with screening or more specific *in vitro* tests. Other parameters have the same values as in parameterization A.

Table 5: Substance-specific parameters used in parameterization C in human. PCAir was set to 1e+99

Table 6: Summary of available *in vivo* data on kinetics. Details are provided in supplementary information, section 6.1

Table 7: Rat and human PBTK parameters for valproic acid.

Table 8: Percentage of VPA concentration predictions within a 10- and 2-fold factor of the observations

Table 9: Percentage of VPA predicted AUC within a 10- and 2-fold factor of the observed AUC

Table 10: Percentage of VPA predicted Cmax within a 10- and 2-fold factor of the observed Cmax

Table 11: Percentage of predictions within a 10-fold factor of the observations

Table 12: Substances for which steady state is not achieved after 100 days or 2000 days continuous exposure in humans