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EuroMix PBPK model for combined exposures

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ABSTRACT

In this paper documents the structure and default parameter values of the EuroMix PBPK model implemented in the MCRA platform for rats and humans. A version allowing apportionment of results by exposure routes is also described. Finally, the extensions needed to describe pharmacokinetic interactions between pairs or triplets of substance are discussed.

INTRODUCTION

Physiologically based pharmacokinetic (PBPK) models are mathematical representations of the animal or human body aimed at describing and predicting the time course distribution of chemicals in tissues and organs. Those internal dose metrics can usefully replace external exposure dose in the derivation of the quantitative dose-response relationships and following risk assessments. PBPK models can simulate both internal doses from exposure scenarios (forward dosimetry) and external dose from biomonitoring data (reverse dosimetry). There is also a growing interest in PBPK models able to compute the contribution of each exposure route to the resulting internal concentrations, and in PBPK models able to simulate the pharmacokinetic interactions occurring during co-exposures to multiple chemicals.

Building a PBPK model requires defining its structural equations and gathering a large amount of data to set its parameter values. We can differentiate system's parameters (physiological, anatomical, biochemical parameters, specific of the animal species considered), and chemical-specific data. We describe here the structure and default parameter values of the “COSMOS” PBPK model implemented in the MCRA simulation platform. We also describe extensions of the model to handle the calculation of routes’ contributions and metabolic interactions between multiple chemicals during co-exposures.

METHODS

PBPK model

We used an updated version (version 6) of the generic PBPK model developed at INERIS in the framework of COSMOS [1] (Figure 1). The model describes the distribution of chemicals in venous blood, arterial blood, adipose tissues, poorly perfused tissues (muscles), gut lumen, 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 [2]. Exposure can occur through the dermal route, ingestion or inhalation. The absorbed molecules can be excreted to urine, exhaled through the lung, or metabolized in liver.

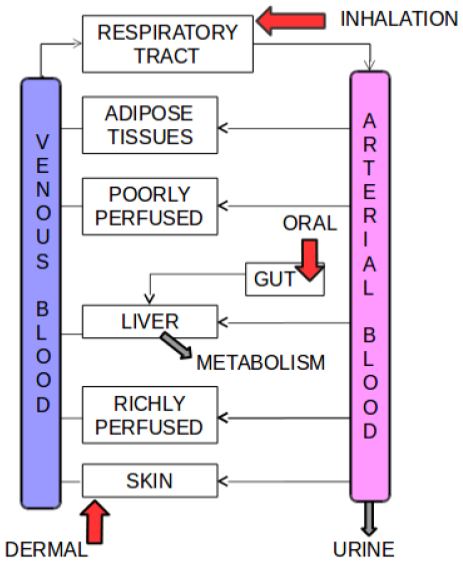

Figure 1: Schematic representation of the “COSMOS” PBPK model implemented in the MCRA platform.

Equations

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 variables). There are 13 state variables in the model: the quantity of chemical in venous blood (Q_{ven}), in arterial blood (Q_{art}), in adipose tissues (Q_{fat}), in poorly perfused tissues (Q_{p}), in well perfused tissues (Q_{r}), in liver (Q_{liv}), in unexposed skin ($Q_{s,u}$), in exposed skin ($Q_{s,e}$), in the stratum corneum of unexposed skin ($Q_{sc,u}$), in exposed stratum corneum ($Q_{sc,e}$), in gut lumen (Q_{gut}), the quantity excreted to urine (Q_{ex}), and the quantity metabolized (Q_{met}). The model can predict, as a function of time, for given oral, dermal and/or inhalation exposures, all the above quantities and the corresponding concentrations as a function of time. Concentrations are obtained by dividing quantities by compartment volumes.

The differential equation for Q_{fat} is:

$$\frac{\partial Q_{fat}}{\partial t} = F_{fat} \cdot \left(C_{art} - \frac{C_{fat}}{PC_{fat}} \right) \quad (1)$$

where F_{fat} is the blood flow to the fat, C_{art} the arterial blood concentration, C_{fat} the concentration in the adipose tissue, and PC_{fat} the fat over blood partition coefficient.

Similar equations are used for the poorly perfused and the richly perfused compartments:

$$\frac{\partial Q_{p}}{\partial t} = F_{p} \cdot \left(C_{art} - \frac{C_{p}}{PC_{p}} \right) \quad (2)$$

$$\frac{\partial Q_{r}}{\partial t} = F_{r} \cdot \left(C_{art} - \frac{C_{r}}{PC_{r}} \right) \quad (3)$$

In the two above equations, the parameters and variables have the same definitions as in Eq. 1, with a change of subscript.

The differential equation for Q_{gut} is:

$$\frac{\partial Q_{gut}}{\partial t} = Frac \times R_{ing} - k_{gut} \times Q_{gut} \tag{4}$$

where *Frac* is the fraction of chemical administered orally that is available for absorption (bioavailability), R_{ing} the ingestion rate (quantity ingested per unit time), and k_{gut} the absorption rate (quantity absorbed through the gut wall per unit time).

The time derivative of the quantity metabolized depends on whether linear or saturable kinetics are assumed:

$$\frac{\partial Q_{met}}{\partial t} = \begin{cases} f_{ub} \times CLH \dfrac{C_{liv}}{PC_{liv}}, & \text{if metabolism is linear} \\ f_{ub} \times V_{liv} \times V_{max} \dfrac{C_{liv}}{PC_{liv} \times K_m + C_{liv}}, & \text{otherwise} \end{cases} \tag{5}$$

where f_{ub} is the fraction of chemical unbound in blood, *CLH* the hepatic metabolic clearance, C_{liv} the concentration in liver, PC_{liv} the liver over blood partition coefficient, V_{liv} the liver volume, V_{max} the maximum rate of metabolism, and K_m the Michaelis-Menten constant for metabolism.

The equation for the liver quantity accounts for blood transport, absorption from the gut lumen and metabolism:

$$\frac{\partial Q_{liv}}{\partial t} = F_{liv} \cdot \left(C_{art} - \frac{C_{liv}}{PC_{liv}} \right) + k_{gut} \times Q_{gut} - \frac{\partial Q_{met}}{\partial t} \tag{6}$$

where F_{liv} is the blood flow to the liver.

The equation for the quantity of chemical in the unexposed fraction of the skin stratum corneum is given by:

$$\frac{\partial Q_{sc,u}}{\partial t} = Kp_{sc,vs} \times BSA \cdot \left(1 - fSA_{exposed}\right) \times \left(\frac{C_{s,u}}{PC_{sc}} - C_{sc,u}\right) \tag{7}$$

where $Kp_{sc,vs}$ is the diffusion rate of the chemical from stratum corneum to viable skin, BSA the body surface area, $fSA_{exposed}$ the fraction of BSA exposed to the chemical, $C_{s,u}$ the concentration of chemical in the unexposed viable skin, PC_{sc} the viable skin over stratum corneum partition coefficient, and $C_{sc,u}$ the concentration of chemical in the unexposed stratum corneum.

The differential equation for quantity in viable skin unexposed is:

$$\frac{\partial Q_{s,u}}{\partial t} = F_{s,u} \cdot \left(C_{art} - \frac{C_{s,u}}{PC_s}\right) - \frac{\partial Q_{sc,u}}{\partial t} \tag{8}$$

where $F_{s,u}$ is the blood flow to the unexposed viable skin, and PC_s the viable skin over blood partition coefficient.

Similarly, for the exposed skin stratum corneum, we have:

$$\frac{\partial Q_{sc,e}}{\partial t} = Kp_{sc,vs} \times BSA \times fSA_{exposed} \cdot \left(\frac{C_{s,e}}{PC_{sc}} - C_{sc,e}\right) + R_{derm} \tag{9}$$

where $C_{s,e}$ the concentration of chemical in the exposed viable skin, $C_{sc,e}$ the concentration in the exposed stratum corneum, and R_{derm} the dermal exposure rate (quantity entering the exposed stratum corneum per unit time).

The differential equation for quantity in viable skin exposed is:

$$\frac{\partial Q_{s,e}}{\partial t} = F_{s,e} \cdot \left(C_{art} - \frac{C_{s,e}}{PC_s}\right) - Kp_{sc,vs} \times BSA \times fSA_{exposed} \cdot \left(\frac{C_{s,e}}{PC_{sc}} - C_{sc,e}\right) \tag{10}$$

where $F_{s,e}$ is the blood flow to the exposed viable skin.

The time evolution quantity of chemical of chemical excreted to urine is governed by:

$$\frac{\partial Q_{ex}}{\partial t} = K_e \times f_{ub} \times C_{art} \tag{11}$$

For the quantity of chemical in arterial blood, we have:

$$\frac{\partial Q_{art}}{\partial t} = F_{alv} \cdot \left(C_{inh} - \frac{C_{art}}{PC_{air}} \right) + F_{blood} \cdot \left(C_{ven} - C_{art} \right) - \frac{\partial Q_{ex}}{\partial t} \tag{12}$$

where C_{inh} the concentration of chemical in the air (*i.e.*, inhaled), and PC_{air} the blood over air partition coefficient.

Finally, for the quantity in venous blood:

$$\frac{\partial Q_{ven}}{\partial t} = \sum_i \left(F_i \cdot \frac{C_i}{PC_i} \right) - F_{blood} \times C_{ven} \tag{13}$$

where the indices i include the compartment indices for fat, poorly perfused, richly perfused, liver, skin exposed, and skin unexposed.

Parameterization

This generic model contains 14 physiological parameters, 15 chemical-dependent parameters (16 parameters if saturable, Michaelis-Menten, metabolism is used), and seven exposure parameters or inputs (those are discussed in the next section).

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 called “scaling coefficients”.

Total blood flow is assumed to be proportional to body mass (*BM*):

$$F_{blood} = scF_{blood} \times BM \tag{14}$$

Blood flows to the individual compartments are scaled in turn to total blood flow (and therefore, indirectly to body mass):

$$F_i = scF_i \times F_{blood} \tag{15}$$

with i belonging to set {*fat, p, liv, s*} (for fat, poorly perfused, liver, and skin, respectively).

To respect summation of individual flows to total flow in case of random sampling, the blood flow to the richly perfused tissues is computed as:

$$F_r = F_{blood} - F_{fat} - F_p - F_{liv} - F_s \tag{16}$$

The blood flows to exposed and unexposed skin are simply apportioned using the fraction of skin surface area exposed (an exposure parameter):

$$F_{s,e} = F_s \times fSA_{exposed} \tag{17}$$

$$F_{s,u} = F_s - F_{s,e} \tag{18}$$

The following compartment volumes are scaled to body mass:

$$V_i = scV_i \times BM \tag{19}$$

with i belonging to set {*fat, r, liv, blood*} (for fat, richly perfused, liver, and blood, respectively).

Arterial blood volume represents a third of total blood, and venous blood the remainder:

$$V_{art} = V_{blood}/3 \tag{20}$$

$$V_{ven} = V_{blood} - V_{art} \tag{21}$$

The volumes of exposed and unexposed skin and skin stratum corneum are simply proportional to the respective surface areas and thicknesses (H_s and H_{sc}):

$$V_{s,e} = BSA \times H_s \times fSA_{exposed} \tag{22}$$

$$V_{s,u} = BSA \times H_s \cdot \left(1 - fSA_{exposed}\right) \tag{23}$$

$$V_{sc,e} = BSA \times H_{sc} \times fSA_{exposed} \tag{24}$$

$$V_{sc,u} = BSA \times H_{sc} \cdot \left(1 - fSA_{exposed}\right) \tag{25}$$

To respect summation of individual compartment volumes to body mass (minus 10% bones) in case of random sampling, the volume of the poorly perfused tissues is computed as:

$$V_p = 0.9 \times BM - V_{fat} - V_r - V_{liv} - V_{s,e} - V_{s,u} - V_{sc,e} - V_{sc,u} - V_{blood} \tag{26}$$

To account for the fact that partition coefficients for a given chemical (in particular when computed with QSAR methods) are typically strongly correlated, they are made proportional to the fat over blood partition coefficient:

$$PC_{fat} = \exp\left(logPC_{fat}\right) \tag{27}$$

$$PC_i = PC_{fat} \times \exp\left(logA_i\right) \tag{28}$$

with $logA_i$ the log of PC_i over PC_{fat} ratio, and i belonging to set {*liv, r, p, s, sc*} (for liver, richly perfused, poorly perfused, viable skin, and stratum corneum, respectively).

In the above equations all tissues densities are assumed to be equal to 1 (hence kilograms translate to liters). The body mass scaling equations are meant to apply *within* a given species. They should not be used for inter-species extrapolations (allometric scaling can be used for that, but it is not implemented in the model and we recommend using species-specific data). The scaling coefficients used in the above equations should be set to meaningful values. When the scaled parameters are physiological (*e.g.*, volumes), their scaling coefficients should be, by default, chemical

independent. They have to be specified for the relevant animal species, and their values are expected to be available from the literature or databases (*e.g.*, *Popgen* http://xnet.hsl.gov.uk/Popgen, *Megen* http://megen.useconnect.co.uk, or in the *R* package *httk* [3,4]). Default values for physiological scaling coefficients and unscaled parameters for (Wistar) rats (see Table 1) and humans (Table 2) have been implemented in MCRA.

The chemical-specific parameters of the model are listed in Table 3. They do not have useful default values and should be set explicitly, using other sources of information, for example the parameter sets provided in the *R* package *httk* [3].

Table 1: Default physiological parameter values or scaling coefficients of the "COSMOS" model proposed in MCRA for Wistar rats.

Parameter	Symbol	Units	Value	References
Body mass	*BM*	kg	0.3	[5,6]
Body skin surface area	*BSA*	dm^2	3.64	[7]
Relative tissue volumes				
Fat	*scVFat*	-	0.073	[5,6]
Richly perfused	*scVRich*	-	0.100	[5,6]
Liver	*scVLiver*	-	0.035	[5,6]
Blood	*scVBlood*	-	0.068	[8]
Relative tissue blood flows				
Fat	*scFFat*	-	0.0054	[5,6]
Poorly perfused	*scFPoor*	-	0.100	[5,6]
Liver	*scFLiver*	-	0.160	[5,6]
Skin	*scFSkin*	-	0.078	[5,6]
Total blood flow	*scFBlood*	L/h/kg	18.8	[8]
Alveolar ventilation rate	*Falv*	L/h	6.35	[6]
Microsomal proteins	*mic*	mg/g liver	45	[9] [a]
Glomerular filtration rate	*GFR*	L/h	0.09	[3] [a]
Stratum corneum thickness	*Hsc*	dm	0.0001	[10]
Viable skin thickness	*Hs*	dm	0.0094	[5,6] [b]

[a] This parameter is not used in the model and is just given for reference.

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

Table 2: Default physiological parameter values or scaling coefficients of the "COSMOS" model proposed in MCRA for humans.

Parameter	Symbol	Units	Value	References
Body mass	*BM*	kg	57, 75 [a]	[8]
Body skin surface area	*BSA*	dm^2	190	[10]
Relative tissue volumes				
Fat	*scVFat*		0.209	[5,6]
Richly perfused	*scVRich*		0.105	[5,6]
Liver	*scVLiver*		0.024	[5,6]
Blood	*scVBlood*		0.068	[8]
Relative tissue blood flows				
Fat	*scFFat*		0.046	[5,6]
Poorly perfused	*scFPoor*		0.134	[5,6]
Liver	*scFLiver*		0.259	[5,6]
Skin	*scFSkin*		0.054	[5,6]
Total blood flow	*scFBlood*	L/h/kg	4.8	[8]
Alveolar ventilation rate	*Falv*	L/h	2220	[6]
Microsomal proteins	*mic*	mg/g liver	52.5 [b]	[9]
Stratum corneum thickness	*Height_sc*	dm	0.0001	[10]
Viable skin thickness	*Height_vs*	dm	0.0122	[5,6] [c]

[a] The first value is for females, the second for males.

[b] This parameter is not used in the model and is just given for reference.

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

Table 3: Chemical-specific parameters of the MCRA "COSMOS" model.

Parameter	Symbol	Unit
Partition coefficient related		
Blood / air p. c.	PC_{Air}	-
Natural log of fat/blood p. c.	$logPC_{Fat}$	-
Liver p. c. scaling factor	$logA_{Liver}$	-
Poorly perfused p. c. scaling factor	$logA_{Poor}$	-
Richly perfused p. c. scaling factor	$logA_{Rich}$	-
Viable skin p. c. scaling factor	$logA_{Skin}$	-
Viable skin p. c. scaling factor	$logA_{Skin\_sc}$	-
Unbound fraction in blood	*fub*	-
Fraction absorbed by gut	*Frac*	-
Oral absorption rate	*kGut*	1/h
Renal excretion rate	*Ke*	L/h
Linear metabolism flag	*Michaelis*	-[a]
Maximum metabolic rate	*Vmax*	mmole/h/L liver
Michaelis-Menten constant	*Km*	mM
Linear metabolic clearance	*CLH*	L/h
Skin diffusion coefficient	*Kp_sc_vs*	dm/h

[a] This indicator variable should be set to zero if metabolism is linear and to one if is saturable. In the first case, *CLH* should be given a value, in the second case *Vmax* and *Km* should be given. If there is no metabolism, *Michaelis* should be set to zero and CLH also.

Exposure modeling

Three routes of exposure, oral, dermal, and inhalation, can be modeled concurrently (multiple routes of exposure for the same chemical). Inhalation is in effect when the inhaled concentration C_{inh} is set to a non-zero value. In that case, a meaningful value of

the PC_{air} partition coefficient should be provided. Note that even when C_{inh} is set to zero, exhalation can occur. To prevent the exit of chemicals from the lung, their partition coefficient should be set to a practically infinite value (*e.g.*, 10^{99}).

Oral exposures can be continuous, with ingestion rate R_{ing} (mmole per hour) going to the gut lumen. For bolus ingestion, the exposure parameter *OralDose* (in mmole) should be set to a non-zero value. In that case, the quantity *OralDose* multiplied by bioavailability *Frac*, will be placed at time zero in the gut lumen.

Dermal exposures can be continuous too, with exposure rate R_{derm} (mmole per hour) going to the skin stratum corneum. For a unique dermal exposure, the exposure parameter *DermalDose* (in mmole) should be set to a non-zero value. In that case, it will be placed at time zero in the stratum corneum.

Contributions of individual exposure routes to model predictions

In the case of multiple exposure routes to a given chemical, the contribution of each one of three exposure routes to any model precited value is easy to compute. In the case of linear metabolism or no metabolism of the chemical considered, all model predicted quantities or concentrations are directly proportional to the sum of exposures. Therefore, the simplest approach, implemented in MCRA, is to first run the model with all routes active, then with only oral exposure, then only dermal exposure, and finally only inhalation exposure. The results obtained with individual exposure routes are simply divided by the corresponding value after multiple exposure to form exact estimates of the fraction contributed by each route.

In the case of saturable (Michaelis-Menten) metabolism, there is no simple proportionality between exposures and prediction, because the various "streams" of molecules coming from different exposure routes compete for metabolism in the liver. This can be modeled as a particular case of metabolic interaction. Therefore, we developed a particular version of the COSMOS model in which model equations 1-3, 6-8, 10-11, and 13 are replicated for each stream of molecules coming from a given exposure route, stream 1 stemming from oral exposure, stream 2 for dermal exposure, and stream 3 from inhalation exposure. This extended model has therefore 39 state variables (noted collectively *Q1*, *Q2*, *Q3* for stream 1, 2, 3 respectively). Equations 4, 5, 9, and 12 were adapted as follows, because they are stream-dependent. No new parameter was needed, because the same parameters apply to the three streams.

The right-hand side of the differential equations for $Q2_{gut}$ (for stream 2) and $Q3_{gut}$ (for steam 3) was set to zero, because there is by definition no oral exposure in those cases. For stream 1 it remains similar to Eq. 4:

$$\frac{\partial Q1_{gut}}{\partial t} = Frac \times R_{ing} - k_{gut} \times Q1_{gut} \tag{29}$$

The time derivative of the quantity metabolized for each stream depends on the other streams in the case of saturable kinetics, because of competition between streams (this is the sole source of non-linearity). In the extended model we therefore have:

$$\frac{\partial Q1_{met}}{\partial t} = \begin{cases} f_{ub} \times CLH \dfrac{C1_{liv}}{PC_{liv}}, & \text{if metabolism is linear} \\ f_{ub} \times V_{liv} \times V_{max} \dfrac{C1_{liv}}{PC_{liv} \cdot K_m + C2_{liv} + C3_{liv} + C1_{liv}}, & \text{otherwise} \end{cases} \tag{30}$$

$$\frac{\partial Q2_{met}}{\partial t}=\begin{cases} f_{ub}\times CLH\dfrac{C2_{liv}}{PC_{liv}}, & \text{if metabolism is linear} \\ f_{ub}\times V_{liv}\times V_{max}\dfrac{C2_{liv}}{PC_{liv}\cdot\left(K_m+C1_{liv}+C3_{liv}\right)+C2_{liv}}, & \text{otherwise} \end{cases} \tag{31}$$

$$\frac{\partial Q3_{met}}{\partial t}=\begin{cases} f_{ub}\times CLH\dfrac{C3_{liv}}{PC_{liv}}, & \text{if metabolism is linear} \\ f_{ub}\times V_{liv}\times V_{max}\dfrac{C3_{liv}}{PC_{liv}\cdot\left(K_m+C1_{liv}+C2_{liv}\right)+C3_{liv}}, & \text{otherwise} \end{cases} \tag{32}$$

The differential equations also differ between streams for the exposed skin stratum corneum, because R_{derm} is only applied to stream 2:

$$\frac{\partial Q1_{sc,e}}{\partial t}=Kp_{sc,vs}\times BSA\times fSA_{exposed}\cdot\left(\frac{C1_{s,e}}{PC_{sc}}-C1_{sc,e}\right) \tag{33}$$

$$\frac{\partial Q2_{sc,e}}{\partial t}=Kp_{sc,vs}\times BSA\times fSA_{exposed}\cdot\left(\frac{C2_{s,e}}{PC_{sc}}-C2_{sc,e}\right)+R_{derm} \tag{34}$$

$$\frac{\partial Q3_{sc,e}}{\partial t}=Kp_{sc,vs}\times BSA\times fSA_{exposed}\cdot\left(\frac{C3_{s,e}}{PC_{sc}}-C3_{sc,e}\right) \tag{35}$$

Similarly, inhalation is only relevant for stream 3, therefore for the quantity of chemical in arterial blood, we have:

$$\frac{\partial Q1_{art}}{\partial t}=-F_{alv}\frac{C1_{art}}{PC_{air}}+F_{blood}\cdot\left(C1_{ven}-C1_{art}\right)-\frac{\partial Q1_{ex}}{\partial t} \tag{36}$$

$$\frac{\partial Q2_{art}}{\partial t}=-F_{alv}\frac{C2_{art}}{PC_{air}}+F_{blood}\cdot\left(C2_{ven}-C2_{art}\right)-\frac{\partial Q2_{ex}}{\partial t} \tag{37}$$

$$\frac{\partial Q3_{art}}{\partial t}=F_{alv}\cdot\left(C_{inh}-\frac{C3_{art}}{PC_{air}}\right)+F_{blood}\cdot\left(C3_{ven}-C3_{art}\right)-\frac{\partial Q3_{ex}}{\partial t} \tag{38}$$

When this model is run, it outputs estimates of quantities and concentrations stemming from each exposure route in each compartment. The relative contributions can then be

obtained by simply dividing the three results for a given compartment at a given time by their sum.

Contributions from individual exposure routes will be illustrated for Imazalil by predicting kinetics after single doses to each route.

Co-exposures modeling

In the case of exposure to multiple chemicals, it may be necessary to take into account their potential competition for access to saturable enzyme-mediated transport or metabolism [11,12]. When no such competitions are expected to occur (different metabolic pathways, linear kinetics, or approximately linear in the case of very low internal concentrations), simulations of exposure to the various chemicals can be performed independently and internal exposure estimates can simply be cumulated afterward.

If competitive metabolic inhibitions may occur between chemicals, it is possible to use a specific version of the COSMOS model which can simulate metabolic interactions by competitive inhibition, between up to three chemicals. The structure of the model is very similar to the one used for calculating route of exposures' contributions: Three sets of differential equations are used, one for each chemical quantity in each compartment. There are then again three times 13, so 39 state variables, which we group in sets *Q1*, *Q2*, *Q3* in the following. The chemical-specific and exposure-related parameters are now different for each chemical (except that the fraction of body surface area exposed should be the same across chemicals). The COSMOS model equations 1-4 and 6-13 are replicated for each chemical. Equation 5, for metabolism is adapted as follows (compare to equations 30 to 32):

$$\frac{\partial Q1_{met}}{\partial t} = \begin{cases} f_{ub1} \times CLH_1 \frac{C1_{liv}}{PC_{liv1}}, & \text{if metabolism is linear} \\ \dfrac{f_{ub1} \times V_{liv} \times V_{max1} \times C1_{liv}}{PC_{liv1} \times K_{m1} \cdot \left(1 + \dfrac{C2_{liv}}{PC_{liv2} \times K_{m2}} + \dfrac{C3_{liv}}{PC_{liv3} \times K_{m3}}\right) + C1_{liv}}, & \text{otherwise} \end{cases} \quad (39)$$

$$\frac{\partial Q2_{met}}{\partial t} = \begin{cases} f_{ub2} \times CLH_2 \frac{C2_{liv}}{PC_{liv2}}, & \text{if metabolism is linear} \\ \dfrac{f_{ub2} \times V_{liv} \times V_{max2} \times C2_{liv}}{PC_{liv2} \times K_{m2} \cdot \left(1 + \dfrac{C1_{liv}}{PC_{liv1} \times K_{m1}} + \dfrac{C3_{liv}}{PC_{liv3} \times K_{m3}}\right) + C2_{liv}}, & \text{otherwise} \end{cases} \quad (40)$$

$$\frac{\partial Q3_{met}}{\partial t} = \begin{cases} f_{ub3} \times CLH_3 \frac{C3_{liv}}{PC_{liv3}}, & \text{if metabolism is linear} \\ \dfrac{f_{ub3} \times V_{liv} \times V_{max3} \times C3_{liv}}{PC_{liv3} \times K_{m3} \cdot \left(1 + \dfrac{C1_{liv}}{PC_{liv1} \times K_{m1}} + \dfrac{C2_{liv}}{PC_{liv2} \times K_{m2}}\right) + C3_{liv}}, & \text{otherwise} \end{cases} \quad (41)$$

RESULTS

Estimating contributions of individual exposure routes

The calculation of contributions from oral, dermal, and inhalation exposure routes to the tissue concentrations is illustrated with predictions for Imazalil in Figure 1 for a 70 kg human. The quantity administered by each route was the same (1 mg), however only 80% of the orally administered dose was absorbed. The concentration in inhaled air was set to 0.054 mg L^{-1} for 1 min. The exposed skin represented 0.26% of body surface area (0.50 dm^2).

The physiological parameters used are given in Table 2, above. Imazalil-specific parameter values are given in Table 4. For some parameters, we had *a priori* information from the literature, or QSAR models (even if imprecise). The others were calibrated using human volunteers data [13].

Dermally administered Imazalil diffuses relatively slowly from exposed skin to the rest of the body: concentrations equilibrate with other those from other routes after approximately half a day.

DISCUSSION

This paper has presented in detail the structure of the COSMOS model as implemented in the MCRA simulation platform. This model is an extended version of the one used in [14,15]. An important extension is the capability to compute the contributions of individual routes of exposures for a particular internal dose. For example, that can be used to estimate what fraction of liver concentration is attributable to oral exposure, and hence whether reducing oral exposures would significantly reduce liver exposure, and potentially hepatotoxicity. This type of calculations is illustrated in the case of hypothetical exposures to Imazalil by the dermal route, the oral route and by inhalation (of an aerosol, for example).

Another extension allows the simulation of co-exposure to different chemicals, inhibiting each other metabolism by a competitive mechanism. We have already demonstrated the use of such models in more complex settings [11,12]. We also used a similar model to estimate the effect of mixtures of aromatase inhibitors (although not necessarily substrates of aromatase) [16]. Note that this version of the COSMOS model does not simulate other mechanisms of metabolic interactions, or interactions arising from the mechanism of toxicity itself.

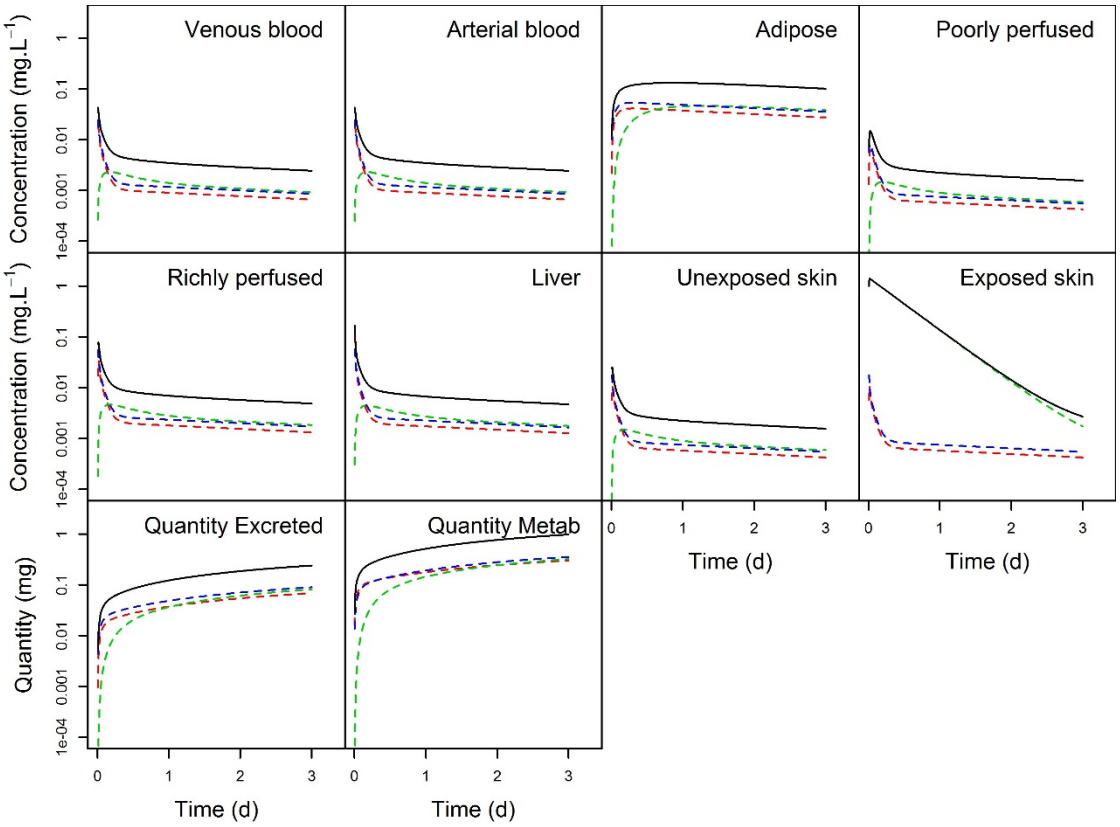

Figure 1: Imazalil concentrations in 8 compartments and total mass excreted and metabolised after a single dose of imazalil administered via 3 routes, 1mg administered orally (red dashed line), 1mg administered dermally (green dashed line), 1 mg administered by inhalation (blue dashed line). Black line: total Imazalil concentrations.

Table 4: Imazalil-specific parameter values used for human multiple routes of exposure simulations.

Parameter	Symbol	Unit	Value	Reference
Partition coefficient related				
Blood / air p. c.	PC_{Air}	-	10^{99}	[17]
Natural log of fat/blood p. c.	$logPC_{Fat}$	-	3.60	[17]
Liver p. c. scaling factor	$logA_{Liver}$	-	-2.90	[17]
Poorly perfused p. c. scaling factor	$logA_{Poor}$	-	-4.04	[17]
Richly perfused p. c. scaling factor	$logA_{Rich}$	-	-2.90	[17]
Viable skin p. c. scaling factor	$logA_{Skin}$	-	-4.04	[17]
Viable skin p. c. scaling factor	$logA_{Skin\_sc}$	-	-7.64	- [a]
Unbound fraction in blood	*fub*	-	0.05	[3][b]
Fraction absorbed by gut	*Frac*	-	0.8	- [b]
Oral absorption rate	*kGut*	1/h	10	- [b]
Renal excretion rate	*Ke*	L/h	17	- [b]
Linear metabolism flag	*Michaelis*	-	0	
Linear metabolic clearance	*CLH*	L/h	70	- [b]
Skin diffusion coefficient	*Kp_sc_vs*	dm/h	0.00001	- [b]

[a] This value was estimated as $logA_{Skin\_sc}$ - $logPC_{Fat}$.

[b] Value adjusted to fit the data (see text and Table 5).

CONCLUSION

The extended COSMOS model can be used for assessment and comparison of internal doses in rats and humans. It can perform exposure sources apportionment and can simulate competitive metabolic interactions between triplets of chemicals. Obviously, the quality of the results will depend on the quality of the data used to define parameter values. New *in vitro* and *in silico*

methods are particularly promising in that respect. The model is available through the MCRA simulation platform.

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