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Generic solving of physiologically-based kinetic models in support of next generation environmental risk assessment

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Contents

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1 Introduction

Need to cite this document: OECD, 2021. Guidance document on the characterisation , validation and reporting of Physiologically Based Kinetic (PBK) models for regulatory purposes. Series on Testing and Assessment No. 331.
Multi-compartment modelling is
Generic solving of a multi-compartment toxicokinetic model [6]
******** to complete ****************************** ******** define TK and PBTK abbreviations *********

1.1 Starting hypotheses

- The exposure concentration is assumed constant over time;
- There can be any number of compartments;
- All compartments are connected two-by-two to all others;
- All compartments are connected to the external medium;
- The exposure concentration is assumed to enter within each compartment.

1.2 Notations

Table 1 below gathers all variables and parameters involved in the generic writing of the toxicokinetic model

1.3 General scheme

Figure 1 below shows the relationship between two given compartments, with all uptake and elimination rates driving the dynamic of the internal concentrations.

Names	**Meaning**	**Unit**
t	time	$[t]$
n	total number of compartments	#
i, j	Compartment numbers	$i, j \in [1; n]$
c_x	exposure concentration in the external medium	mass per volume
$c_i(t)$	internal concentration in compartment i at time t	mass per weight
$k_{u,i}$	uptake rate from the external medium to compartment i	$[t]^{-1}$
$k_{e,i}$	elimination rate from compartment i to the external medium	$[t]^{-1}$
$k_{i,j}$	input rate from compartment j to compartment i	$[t]^{-1}$
$k_{j,i}$	output rate from compartment i to compartment j	$[t]^{-1}$

Table 1: Variable and parameter names, meaning and unit as used within the generic toxicokinetic model all a long this paper. Symbol # means dimensionless; $[t]$ stands for time unit.

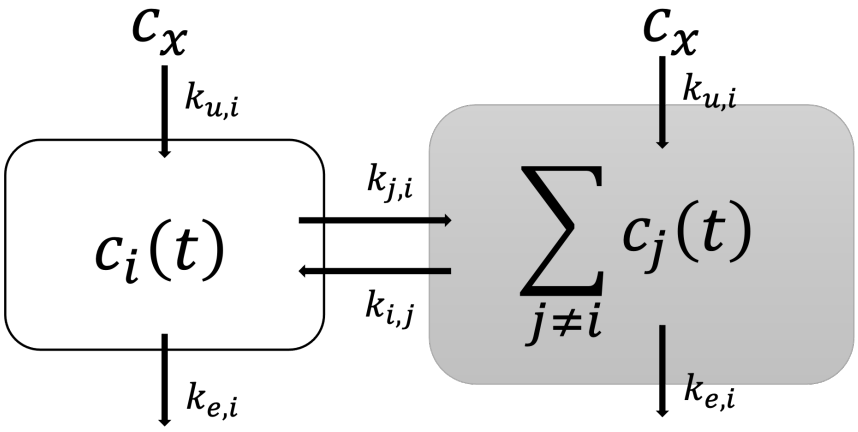

Figure 1: Generic scheme of a multi-compartment toxicokinetic model connecting n compartments two-by-two and each of them to the external medium at exposure concentration c_x. Refer to Table 1 for names, meaning and unit of variables and parameters.

1.4 Mathematical equations

Below is the full system of ordinary differential equation (ODE) describing the dynamics of the multi-compartment model when organisms are exposed to the external concentration c_x:

$$\frac{dc_i(t)}{dt} = k_{u,i}c_x - k_{e,i}c_i(t) + \sum_{j\neq i} k_{i,j}C_j(t) - \left(\sum_{j\neq i} k_{j,i}\right) c_i(t) \quad \forall i,j \in [1;n] \tag{1}$$

Names, meaning and units of variables and parameters are provided in Table 1.
This full system of ODE for n compartments all related by pairs (equation 1) can equivalently be written in a matrix way as follows:

$$\frac{d\mathbf{C}(t)}{dt} = \mathbf{U}\, c_x + \mathbf{E}\, \mathbf{C}(t) \tag{2}$$

where vector $\mathbf{C}(t)$ gathers all internal concentrations in compartments i at time t, $i \in [1;n]$:

$$\mathbf{C}(t) = \begin{pmatrix} c_1(t) & c_2(t) & \dots & c_n(t) \end{pmatrix}^T \tag{3}$$

Vector $\mathbf{U}$ contains all uptake rates from the external medium at exposure concentration c_x:

$$\mathbf{U} = \begin{pmatrix} k_{u,1} & k_{u,2} & \dots & k_{u,n} \end{pmatrix}^T \tag{4}$$

Matrix $\mathbf{E}$ gathers both input and output rates between compartments two-by-two, together with the elimination rates from each compartment i, $i \in [1;n]$:

$$\mathbf{E} = [e_{ij}]_{i,j\in[1;n]} \quad \text{with} \quad \begin{cases} e_{ii} = -k_{e,i} - \sum_{j\neq i} k_{j,i} \\ e_{ij} = k_{i,j} \end{cases} \tag{5}$$

2 Generic solving

Equation 2 is a matrix ODE, which is linear with a second member. Its solving can be performed in two steps: solving the ODE without its second member (subsection 2.1); then using the method of the variation of constant in order to find the final general solution (subsection 2.2). These steps are detailed below.

2.1 Solving the ODE system without the second member

$$\frac{d\mathbf{C_{wosm}}(t)}{dt} = \mathbf{E}\, \mathbf{C_{wosm}}(t) \tag{6}$$

with $\mathbf{C_{wosm}}(t)$ the desired solution of equation 6. Using matrix exponential immediately provides the following solution:

$$\mathbf{C_{wosm}}(t) = e^{t\mathbf{E}}\mathbf{\Omega_1} \tag{7}$$

with $\mathbf{\Omega_1}$ a vector integration constant ($\in \mathbb{R}^n$), and

$$e^{t\mathbf{E}} = \sum_{j=0}^{\infty} \frac{1}{j!} (t\mathbf{E})^j \tag{8}$$

In theory, $e^{t\mathbf{E}}$ can always be obtained from the Jordan normal form $\mathbf{J}$ associated to $\mathbf{E}$ that can be written as

$$\mathbf{PJP^{-1}} \tag{9}$$

where matrix $\mathbf{P}$ is defined with columns equal to eigenvectors of matrix $\mathbf{E}$.
Matrix $\mathbf{J}$ is a block diagonal matrix formed of Jordan blocks, that are themselves matrix blocks composed of zeroes everywhere except on the diagonal, filled with a fixed element $\lambda \in R$, and the upper-diagonal, filled with ones. Elements λ are the eigenvalues of matrix $\mathbf{E}$, associated with the eigenvectors of matrix $\mathbf{E}$ as used to build matrix $\mathbf{P}$. It follows that:

$$e^{t\mathbf{E}} = \mathbf{Pe^{tJ}P^{-1}} \tag{10}$$

2.2 Getting the generic solution of the ODE system

For the second step of the ODE system solving including the second member, we use the method of the variation of the constant, starting from the hypothesis that the general solution can be written as follows:

$$\mathbf{C_{wsm}}(t) = e^{t\mathbf{E}}\mathbf{\Omega_1}(t) \tag{11}$$

Given that the exposure concentration is constant (equal to c_x, deriving equation 11 and replacing terms in equation 2 leads to the following result:

$$\frac{d\mathbf{\Omega_1}(t)}{dt} = e^{-t\mathbf{E}}\mathbf{U}c_x \Leftrightarrow \mathbf{\Omega_1}(t) = \left(\int_0^t e^{-\tau\mathbf{E}}d\tau\right)\mathbf{U}c_x + \Omega_2 \tag{12}$$

The final generic solution of equation 2 will thus write as follows:

$$\mathbf{C_{wsm}}(t) = \left(\int_0^t e^{(t-\tau)\mathbf{E}}d\tau\right)\mathbf{U}c_x + e^{t\mathbf{E}}\mathbf{\Omega_2} \tag{13}$$

with $\mathbf{\Omega_2} \in \mathbb{R}^n$ a constant to determine. From the initial condition $\mathbf{C_{wsm}}(t) = \mathbf{0}$, we finally get $\mathbf{\Omega_2} = \mathbf{0}$, what corresponds to the following final particular solution of equation 2:

$$\boxed{\mathbf{C_{wsm}}(t) = \left(\int_0^t e^{(t-\tau)\mathbf{E}}d\tau\right)\mathbf{U}c_x} \tag{14}$$

3 Application to bioaccumulation testing

Bioaccumulation is defined as an increase in contaminant concentrations inside living organisms following uptake from the surrounding medium (living media, food, even workplace for humans). Bioaccumulation is the result of dynamical processes of uptake and elimination that can be modelled with the above ODE system (equation 2). The extent to which bioaccumulation occurs determines the subsequent toxic effects. hence, a better knowledge on bioaccumulation enables to assess the risk related with the exposure to chemicals and to evaluate our ability to control their use and emissions in field [4].

Bioaccumulation is thus the net result of all uptake and elimination processes, such as respiratory and dietary uptake, as well as elimination by egestion, passive diffusion, metabolisation, transfer to offspring and growth (Figure ***). Accordingly, bioaccumulation comprises both bioconcentration

and biomagnification. Bioconcentration is the process of direct balance of chemicals between the exposure medium and the organism leading to increased internal concentrations. Biomagnification results in contaminant uptake from the diet leading to higher concentrations in the feeder than in the diet itself. Biomagnification leads to increased internal chemical concentration usually at higher trophic level within the food web [16, 2].

Bioaccumulation tests are usually mid-to-long term laboratory experiments designed to identify all the potential uptake pathways, including food and waterborne exposure routes [9]. Bioaccumulation tests are often called bioconcentration tests when only single route laboratory experiments are designed to obtain information concerning the ability of aquatic species to accumulate chemicals directly from water [11]. Sediment bioaccumulation tests are usually performed to determine the bioavailability of sediment contaminants and to assess their hazard through the trophic chain [10].

All bioaccumulation tests comprise an accumulation phase followed by a depuration phase [5]. During the accumulation phase, organisms are exposed to a chemical substance of interest. After a certain time period (for $t \in [0; t_c]$), with t_c fixed by the experimental design, organisms are transferred to a clean medium for the depuration phase (for $t > t_c$). The concentration of the chemical substance (and of its potential metabolites) within organisms is then measured internally at regular time points during both phases leading *in fine* to the calculation of bioaccumulation metrics [15].

If the bioaccumulation within organisms is widely studied for humans and large animals, namely, fish, birds, and farm animals [19, 7], this is not the case for smaller species, such as invertebrates [1]. However, it is equally important to decipher internal processes at the target organ level in these species, in order to better understand mechanisms implied in the subsequent effects on their fitness. Among species of interest, crustacean amphipods appears particularly promising as aquatic biomonitors of trace metals for example [1]. It is also of great importance to more deeply unravel internal routes of chemical substances between organs, after they entered within the body, a phenomenon known as organotropism [8, 17, 3].

Within this context, the generic ODE system (equation 2) may fully be applied to describe, simulate and predict what happens within and between organs of an organism when it is exposed to a given chemical substance. To this end, each organ can be associated with one model compartment, leading to the following equations for both the accumulation and the depuration phases:

Accumulation phase ($0 \leqslant t \leqslant t_c$)

$$\frac{d\mathbf{C_A}(t)}{dt} = \mathbf{U}\, c_x + \mathbf{E}\, \mathbf{C_A}(t) \tag{15}$$

Equation 15 is identical to equation 2, denoting $\mathbf{C_A}(t)$ the internal concentration at time t during the accumulation phase.

Depuration phase ($t > t_c$)

$$\frac{d\mathbf{C_D}(t)}{dt} = \mathbf{E}\, \mathbf{C_D}(t) \tag{16}$$

Variable $\mathbf{C_D}(t)$ is the internal concentration at time t during the depuration phase. Parameters and variables have the same meaning as given in Table 1.

Regarding the accumulation phase, equation 15 has a solution directly given by equation 14, the initial condition being identical. As a consequence, the generic solution for the accumulation phase writes as follows:

$$\mathbf{C_A}(t) = \left(\int_0^t e^{(t-\tau)\mathbf{E}} d\tau \right) \mathbf{U} c_x \tag{17}$$

Regarding the depuration phase, equation 16 is similar to equation 6, with the corresponding generic solution given by equation 7. The constant vector $\mathbf{\Omega_1}$ can be determined from the initial condition of the depuration phase that corresponds to the internal concentration reached at $t = t_c$, at the end of the accumulation phase. We must therefore solve the following equation:

$$\mathbf{C_A}(t_c) = \mathbf{C_D}(t_c) \tag{18}$$

Given solutions from equations 14 and equation 7, we get:

$$\mathbf{C_A}(t_c) = \left(\int_0^{t_c} e^{(t_c-\tau)\mathbf{E}} d\tau \right) \mathbf{U} c_x \quad \text{and} \quad \mathbf{C_D}(t_c) = e^{t_c \mathbf{E}} \mathbf{\Omega_1} \tag{19}$$

Hence, the constant vector $\mathbf{\Omega_1}$ will derived from the following equation:

$$\left(\int_0^{t_c} e^{(t_c-\tau)\mathbf{E}} d\tau \right) \mathbf{U} c_x = e^{t_c \mathbf{E}} \mathbf{\Omega_1} \Leftrightarrow \mathbf{\Omega_1} = \left(\int_0^{t_c} e^{-\tau \mathbf{E}} d\tau \right) \mathbf{U} c_x \tag{20}$$

The generic solution for the depuration phase then writes as follows:

$$\mathbf{C_D}(t) = \left(\int_0^{t_c} e^{(t-\tau)\mathbf{E}} d\tau \right) \mathbf{U} c_x \tag{21}$$

4 Case studies in ecotoxicology

This section presents three case studies with different numbers of compartments, all concerning the species *Gammarus fossarum* exposed to cadmium (Cd) as studied in [6]. Indeed, [6] used one- and multi-compartment TK models to gain knowledge on the accumulation and fate dynamic of Cd in and between gammarids organs. Subsections 4.1 and 4.2 give the generic solutions of parameter estimates. Subsection 4.3 presents a five-compartment model currently explored to account for accumulation and depuration also in gills of gammarids (Gestin *et al.*, in prep.).

4.1 Case study with one compartment

Applying equation 2 with only one compartment leads to the two single equation:

$$\begin{cases} \frac{dc_i(t)}{dt} = k_{u,1} c_x - k_{e,1} c_i(t) & \text{when} \quad 0 \leqslant t \leqslant t_c \quad \text{(accumulation phase)} \\ \frac{dc_i(t)}{dt} = -k_{e,1} c_i(t) & \text{when} \quad t > t_c \quad \text{(depuration phase)} \end{cases} \tag{22}$$

Equation 22 can easily be solved with the method of the separation of variables (also known as the Fourier method):

$$\begin{cases} c_i(t) = \frac{k_{u,i}}{k_{e,i}} c_x \left(1 - e^{-k_{e,i} t}\right) & \text{when} \quad 0 \leqslant t \leqslant t_c \quad \text{(accumulation phase)} \\ c_i(t) = \frac{k_{u,i}}{k_{e,i}} c_x \left(e^{k_{e,i}(t_c - t)} - e^{-k_{e,i} t}\right) & \text{when} \quad t > t_c \quad \text{(depuration phase)} \end{cases} \tag{23}$$

Getting solutions of the set of equations 22 directly from the generic expressions given by the combination of both equations 17 and 21 leads exactly to the same result. Indeed, with one compartment, matrix $\mathbf{E} = -k_{e,i}$ and vector $\mathbf{U} = k_{u,i}$. Additionally, integrals in the expressions of $\mathbf{C_A}(t)$ and $\mathbf{C_D}(t)$ write as follows:

Organ	Parameter	Median value (in $[t]^{-1}$)
Intestines	$k_{u,1}$	1917
Caeca	$k_{u,2}$	1571
Cephalons	$k_{u,3}$	91.1
Remaining tissues	$k_{u,4}$	135
Intestines	$k_{e,1}$	0.506
Caeca	$k_{e,2}$	0.053
Cephalons	$k_{e,3}$	0.060
Remaining tissues	$k_{e,4}$	0.026

Table 2: Medians of parameters estimated from TK one-compartment models separately fitted to each organ of *Gammarus fossarum* exposed to dissolved Cd at 11.1 $\mu g.L^{-1}$ for 7 days, before being placed for 14 days under depuration conditions.

$$\int_0^t e^{(t-\tau)\mathbf{E}} d\tau = \frac{1}{k_{e,i}} \left(1 - e^{-k_{e,i}t}\right) \quad \text{and} \quad \int_0^{t_c} e^{(t-\tau)\mathbf{E}} d\tau = \frac{1}{k_{e,i}} \left(e^{k_{e,i}(t_c-t)} - e^{-k_{e,i}t}\right) \tag{24}$$

This finally provides a similar set of ODE than equations 23.

Inspired from [6], by considering only solid black arrows in Figure 2 highlights the target organs that can correspond to one compartment according to i: intestine ($i = 1$); cephalon ($i = 2$); caecum ($i = 3$); remaining tissues ($i = 4$). Each compartment has its own parameter pair for uptake ($k_{u,i}$) and elimination ($k_{e,i}$) rates.

In [6], model parameters were estimated under the unified Bayesian framework proposed by [14]. In particular, parameters TK one-compartment models were fitted separately for each organ of *G. fossarum* exposed to dissolved Cd at 11.1 $\mu g.L^{-1}$ for 7 days, before being placed for 14 days under depuration conditions. Getting median parameter values as given in Table 12 allows to simulate what for example happens within intestines when is connected from all other organs (see Appendix 5.1).

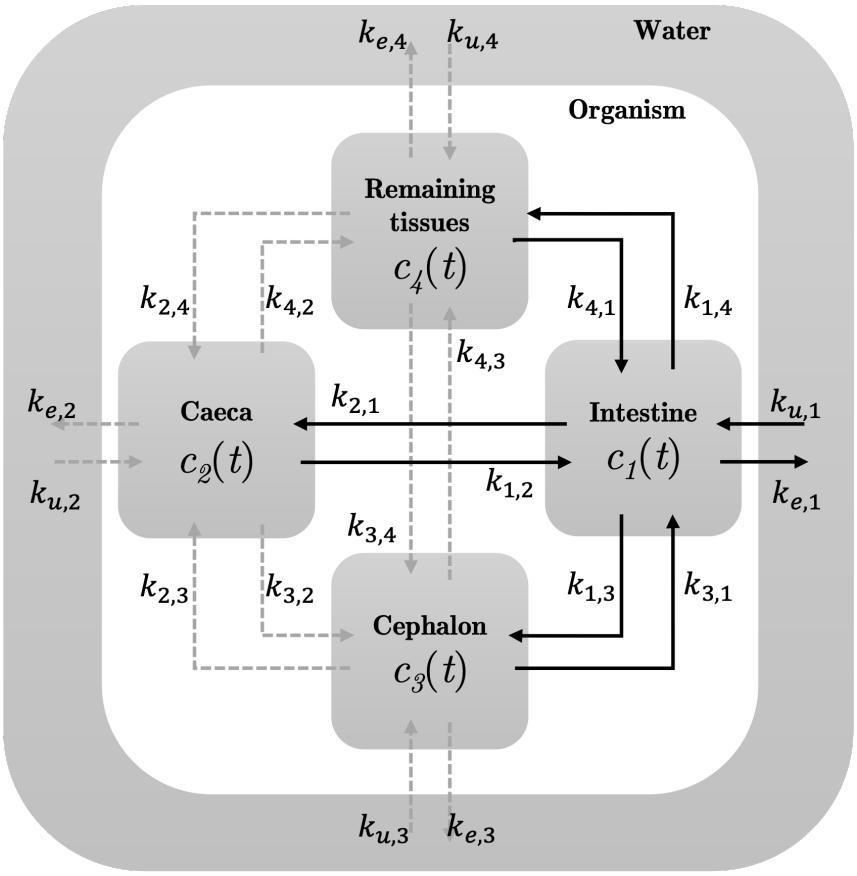

Figure 2: General schema of the multi-compartment toxicokinetic model used by [6] at the initial modelling stage when all compartments were connected to each other.

4.2 Case study with four compartments

Applying the general matrix ODE system given by the set of equations 15 and 16 to the particular case of four compartments connected by pairs (Figure 2) leads to the following writing:

$$\begin{cases} \frac{d\mathbf{C_A}(t)}{dt} = \mathbf{U}\,c_x + \mathbf{E}\,\mathbf{C_A}(t) & \text{when} \quad 0 \leqslant t \leqslant t_c \quad \text{(accumulation phase)} \\ \frac{d\mathbf{C_D}(t)}{dt} = \mathbf{E}\,\mathbf{C_D}(t) & \text{when} \quad t > t_c \quad \text{(depuration phase)} \end{cases} \tag{25}$$

with vectors and matrices defined as follows:

$$\mathbf{C_A}(t) = \mathbf{C_D}(t) = \begin{pmatrix} c_1(t) \\ c_2(t) \\ c_3(t) \\ c_4(t) \end{pmatrix} \quad \mathbf{U} = \begin{pmatrix} k_{u,1} \\ k_{u,2} \\ k_{u,3} \\ k_{u,4} \end{pmatrix} \quad \mathbf{E} = \begin{pmatrix} e_{1,1} & k_{1,2} & k_{1,3} & k_{1,4} \\ k_{2,1} & e_{2,2} & k_{2,3} & k_{2,4} \\ k_{3,1} & k_{3,2} & e_{3,3} & k_{3,4} \\ k_{4,1} & k_{4,2} & k_{4,3} & e_{4,4} \end{pmatrix} \tag{26}$$

and diagonal elements of matrix $\mathbf{E}$ defined by:

$$\begin{cases} e_{1,1} = -k_{e,1} - (k_{2,1} + k_{3,1} + k_{4,1}) \\ e_{2,2} = -k_{e,2} - (k_{1,2} + k_{3,2} + k_{4,2}) \\ e_{3,3} = -k_{e,3} - (k_{1,3} + k_{2,3} + k_{4,3}) \\ e_{4,4} = -k_{e,4} - (k_{1,4} + k_{2,4} + k_{3,4}) \end{cases} \tag{27}$$

The exact solution of the above-mentioned matrix ODE system is given by equations 17 and 21:

$$\begin{cases} \mathbf{C_A}(t) = \left(\int_0^t e^{(t-\tau)\mathbf{E}} d\tau \right) \mathbf{U} c_x \quad \text{when} \quad 0 \leqslant t \leqslant t_c \quad \text{(accumulation phase)} & \text{(28a)} \\ \mathbf{C_D}(t) = \left(\int_0^{t_c} e^{(t-\tau)\mathbf{E}} d\tau \right) \mathbf{U} c_x \quad \text{when} \quad t > t_c \quad \text{(depuration phase)} & \text{(28b)} \end{cases}$$

Given that matrix $\mathbf{E}$ can be decomposed into its Jordan normal form, we get:

$$\mathbf{E} = \mathbf{P}\mathbf{J}\mathbf{P^{-1}}$$

where matrix $\mathbf{J} = diag\left\{\lambda_i\right\}_{i=1,4}$ with λ_i, $i = 1, 4$, the eigenvalues of matrix $\mathbf{E}$; and matrix $\mathbf{P}$ is built binding the eigenvectors associated to each eigenvalue as columns. Because eigenvectors are forming a base, matrix $\mathbf{P}$ is always invertible.
Based on the calculation of a matrix exponential, we get:

$$e^{(t-\tau)\mathbf{E}} = \mathbf{P} e^{(t-\tau)\mathbf{J}} \mathbf{P^{-1}} \Leftrightarrow \left(\int_0^t e^{(t-\tau)\mathbf{E}} d\tau \right) = \mathbf{P} \left(\int_0^t e^{(t-\tau)\mathbf{J}} d\tau \right) \mathbf{P^{-1}}$$

where $e^{(t-\tau)\mathbf{J}} = diag\left\{e^{(t-\tau)\lambda_i}\right\}_{i=1,4}$.

For each compartment i, we can calculate:

$$\int_0^t e^{(t-\tau)\lambda_i} d\tau = \frac{1}{\lambda_i} \left(e^{\lambda_i t} - 1 \right)$$

what finally leads to the following writing of the exact solution for both the accumulation and the depuration phases, respectively:

$$\begin{cases} \mathbf{C_A}(t) = \mathbf{P}\, diag\left\{ \frac{1}{\lambda_i} \left(e^{\lambda_i t} - 1 \right) \right\}_{i=1,4} \mathbf{P^{-1}} \mathbf{U} c_x \quad \text{when} \quad 0 \leqslant t \leqslant t_c & \text{(29a)} \\ \mathbf{C_D}(t) = \mathbf{P}\, diag\left\{ \frac{1}{\lambda_i} e^{\lambda_i t} \left(1 - e^{-\lambda_i t_c} \right) \right\}_{i=1,4} \mathbf{P^{-1}} \mathbf{U} c_x \quad \text{when} \quad t > t_c & \text{(29b)} \end{cases}$$

Developing the previous matrix ODE system given by equations 30 and 31 finally provides the following set of equations below:

- For the accumulation phase ($0 \leqslant t \leqslant t_c$):

$$\begin{cases} \frac{dc_1(t)}{dt} = k_{u,1} c_x - k_{e,1} c_1(t) + k_{1,2} c_2(t) + k_{1,3} c_3(t) + k_{1,4} c_4(t) - (k_{2,1} + k_{3,1} + k_{4,1}) c_1(t) \\ \frac{dc_2(t)}{dt} = k_{u,2} c_x - k_{e,2} c_2(t) + k_{2,1} c_1(t) + k_{2,3} c_3(t) + k_{2,4} c_4(t) - (k_{1,2} + k_{3,2} + k_{4,2}) c_2(t) \\ \frac{dc_3(t)}{dt} = k_{u,3} c_x - k_{e,3} c_3(t) + k_{3,1} c_1(t) + k_{3,2} c_2(t) + k_{3,4} c_4(t) - (k_{1,3} + k_{2,3} + k_{4,3}) c_3(t) \\ \frac{dc_4(t)}{dt} = k_{u,4} c_x - k_{e,4} c_4(t) + k_{4,1} c_1(t) + k_{4,2} c_2(t) + k_{4,3} c_3(t) - (k_{1,4} + k_{2,4} + k_{3,4}) c_4(t) \end{cases} \tag{30}$$

- For the depuration phase ($t > t_c$):

$$\begin{cases} \frac{dc_1(t)}{dt} = -k_{e,1}c_1(t) + k_{1,2}c_2(t) + k_{1,3}c_3(t) + k_{1,4}c_4(t) - (k_{2,1} + k_{3,1} + k_{4,1})c_1(t) \\ \frac{dc_2(t)}{dt} = -k_{e,2}c_2(t) + k_{2,1}c_,(t) + k_{2,3}c_3(t) + k_{2,4}c_4(t) - (k_{1,2} + k_{3,2} + k_{4,2})c_2(t) \\ \frac{dc_3(t)}{dt} = -k_{e,3}c_3(t) + k_{3,1}c_1(t) + k_{3,2}c_2(t) + k_{3,4}c_4(t) - (k_{1,3} + k_{2,3} + k_{4,3})c_3(t) \\ \frac{dc_4(t)}{dt} = -k_{e,4}c_4(t) + k_{4,1}c_1(t) + k_{4,2}c_2(t) + k_{4,3}c_3(t) - (k_{1,4} + k_{2,4} + k_{3,4})c_4(t) \end{cases} \quad (31)$$

This four compartment model, based on the ODE matrix system 25 and the corresponding exact solution 28a and 28b, is assuming that all compartments are connected according to Figure 2 considering all incoming and outgoing arrows from all compartments. This model comprises a total of 20 parameters, plus c_x for the exposure concentration. Median parameter values as given in Table 3 were used to simulate what happens within each organ in terms of internal concentration dynamic, and compared to the previous results with the four independent one-compartment TK models. See Annex 5.2 for details.

4.3 Case study with five compartments

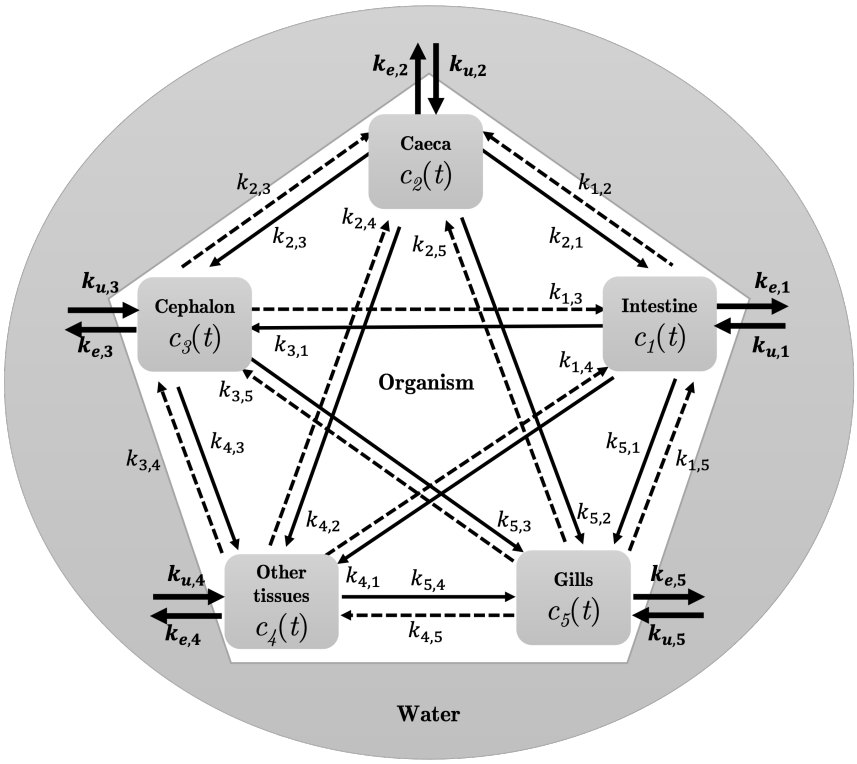

Figure 3: General scheme of the five-compartment toxicokinetic model.

References

[1] M. Amyot, B. Pinel-Alloul, P. G. Campbell, and J. C. Delsy. Total metal burdens in the freshwater amphipod *Gammarus fasciatus*: Contribution of various body parts and influence

of gut contents. *Freshwater Biology*, 35(2):363–373, 1996. ISSN 00465070. doi: 10.1046/j.1365-2427.1996.00493.x.

[2] K. Borga. Bioaccumulation. In S. E. Jorgensen and B. D. Fath, editors, *Encyclopedia of Ecology*, pages 346–348. Academic Press, Oxford, 2008. doi: https://doi.org/10.1016/B978-008045405-4.00374-8.

[3] W. Chen, A. D. Hoffmann, H. Liu, and X. Liu. Organotropism: new insights into molecular mechanisms of breast cancer metastasis. *Precision Oncology*, 2(1), 2018. doi: 10.1038/s41698-018-0047-0.

[4] K. Chojnacka and M. Mikulewicz. Bioaccumulation. In P. Wexler, editor, *Encyclopedia of Toxicology (Third Edition)*, pages 456–460. Academic Press, Oxford, third edition edition, 2014. doi: https://doi.org/10.1016/B978-0-12-386454-3.01039-3.

[5] European Commission. European Commission (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection produc, 2013.

[6] O. Gestin, T. Lacoue-labarthe, M. Coquery, N. Delorme, L. Garnero, L. Dherret, O. Geffard, and C. Lopes. One and multi-compartments toxico-kinetic modeling to understand metals ' organotropism and fate in *Gammarus fossarum*. *Environment international*, 156(April):1–9, 2021. doi: 10.1016/j.envint.2021.106625.

[7] A. Grech, C. Tebby, C. Brochot, F. Y. Bois, A. Bado-Nilles, J. L. Dorne, N. Quignot, and R. Beaudouin. Generic physiologically-based toxicokinetic modelling for fish: Integration of environmental factors and species variability. *Science of the Total Environment*, 651:516–531, 2019. doi: 10.1016/j.scitotenv.2018.09.163.

[8] Y.-R. Ju, W.-Y. Chen, S. Singh, and C.-M. Liao. Trade-offs between elimination and detoxification in rainbow trout and common bivalve molluscs exposed to metal stressors. *Chemosphere*, 85(6):1048–1056, 2011. doi: https://doi.org/10.1016/j.chemosphere.2011.07.033.

[9] I. Marigomez. Environmental risk assessment, marine. In P. Wexler, editor, *Encyclopedia of Toxicology (Third Edition)*, pages 398–401. Academic Press, Oxford, third edition edition, 2014. doi: https://doi.org/10.1016/B978-0-12-386454-3.00556-X.

[10] Organisation for Economic Co-operation and Development. *Test No 315: Bioaccumulation in sediment-dwelling benthic oligochaetes*, volume 315. OECD Publishing, 2008. doi: 10.1787/2074577x.

[11] Organisation for Economic Co-operation and Development. *Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure*, volume 305. OECD Publishing, 2012. doi: 10.1787/9789264185296-en.

[12] L. Petzold. Automatic selection of methods for solving stiff anf nonstiff systems of ODEs.pdf, 1983.

[13] L. Petzold and A. Hindmarsh. A systematized collection of ode solvers. *Report of*, 1997.

[14] A. Ratier, C. Lopes, P. Labadie, N. Budzinski, Hélène Delorme, H. Quéau, L. Peluhet, O. Geffard, and M. Babut. A unified Bayesian framework for estimating model parameters for the bioaccumulation of organic chemicals by benthic invertebrates: proof of concept with PCB153 and two freshwater species. *Ecotoxicology and Environmental Safety*, 180:33–42, 2019.

[15] A. Ratier, C. Lopes, G. Multari, V. Mazerolles, P. Carpentier, and S. Charles. New perspectives on the calculation of bioaccumulation metrics for active substances in living organisms. *Integrated Environmental Assessment and Management*, 18(1):10–18, 2021. doi: 10.1101/2020.07.07.185835.

[16] H. T. Ratte. Bioaccumulation and toxicity of silver compounds: A review. *Environmental Toxicology and Chemistry*, 18(1):89–108, 1999. doi: 10.1002/etc.5620180112.

[17] T. L. Rocha, T. Gomes, J. P. Pinheiro, V. S. Sousa, L. M. Nunes, M. R. Teixeira, and M. J. Bebianno. Toxicokinetics and tissue distribution of cadmium-based quantum dots in the marine mussel *Mytilus galloprovincialis*. *Environmental Pollution*, 204:207–214, 2015. ISSN 0269-7491. doi: https://doi.org/10.1016/j.envpol.2015.05.008.

[18] K. Soetaert, T. Petzoldt, and R. W. Setzer. Solving differential equations in R: Package deSolve. *Journal of Statistical Software*, 33(9):1–25, 2010. doi: 10.18637/jss.v033.i09.

[19] J. V. Tarazona, C. Rodriguez, E. Alonso, M. Saez, F. Gonzalez, M. D. San Andres, B. Jimenez, and M. I. San Andres. Toxicokinetics of perfluorooctane sulfonate in birds under environmentally realistic exposure conditions and development of a kinetic predictive model. *Toxicology Letters*, 232(2):363–368, jan 2015. doi: 10.1016/j.toxlet.2014.11.022.

5 Annexes: R command lines and simulation results

```
# Clean working space
rm(list = ls())
# Load packages is needed
# library(rbioacc)
```

5.1 One-compartment TK model

```
# Write the one-compartment TK model
maccu <- function(x, cx, ku, ke){ # Accumulation phase
    caccu <- (ku * cx / ke) * (1 - exp(- ke * x))
    return(caccu / 1000)
    }
mdepu <- function(x, cx, ku, ke){ # Depuration phase
    cdepu <- (ku * cx / ke) * (exp(ke * (tacc - x)) - exp(- ke * x))
    return(cdepu / 1000)
    }
```

```
# Simulations for one single compartment
par(mar = c(4, 5, 0.1, 0.1), mfrow = c(1, 1))
cx <- 11.1
tacc <- 7
tfin <- tacc + 14
# Intestines
ku <- 1917
ke <- 0.506
curve(maccu(x, cx, ku, ke), from = 0, to = tacc,
    xlim = c(0, tfin), las = 1, lwd = 2, xlab = "Time (days)",
    ylab = expression(paste("[Cd] (in ",mu,"g.",g-1," d.w.)")))
curve(mdepu(x, cx, ku, ke), from = tacc, to = tfin,
    lwd = 2, add = TRUE)
abline(v = tacc, lty = 2)
legend("topright", legend = "(1)", bty = "n")
```

```
# Simulations for all compartments, separately
```

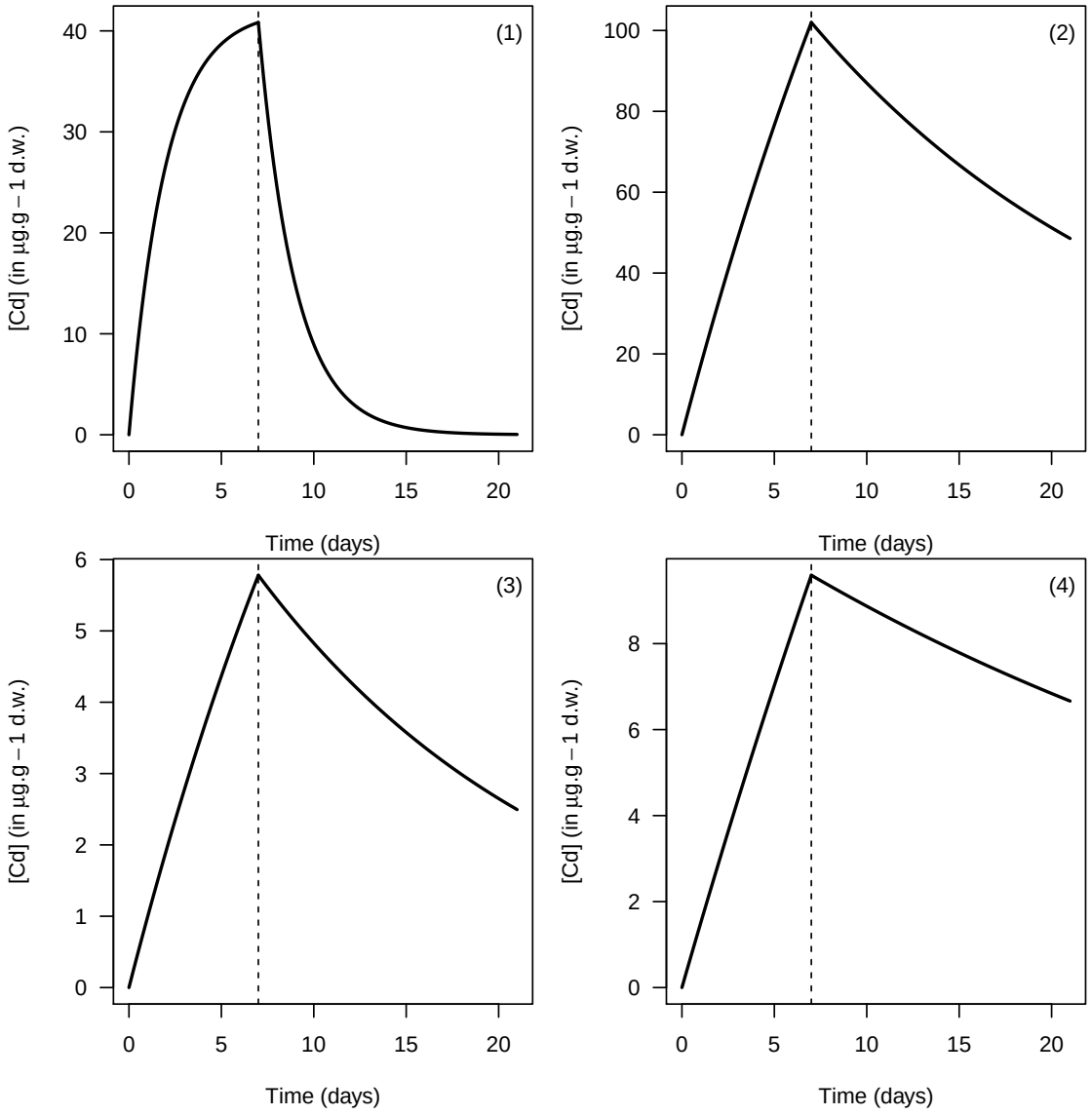

Figure 4: Simulation of bioaccumulation within *Gammarus fossarum* exposed to Cd at concentration 11.1 $\mu g.L^{-1}$. The solid lines stand for simulated internal concentrations; vertical dotted lines delimit accumulation from depuration phases, in intestines ($i = 1$), caeca ($i = 2$); Cephalons ($i = 3$), an remaining tissues ($i = 4$).

5.2 Four-compartment TK model

5.2.1 Connecting all compartments by pairs

We compared two types of simulations: (1) simulation from the exact solution as given by both matrix equations 17 and 21; (2) simulations based on the numerical integration of the ODE system of four equations, with the R-package `'deSolve'` [18], function `'ode()'` [13, 12].

Parameter estimates used to simulate the four-compartments model when all compartment are connected by pairs are given in Table 3 below. Simulations are only based on medians values.

Organ	Parameter	Mean	Median	2.5% quantile	97.5% quantile
Intestines	$k_{u,1}$	2600	1900	0.014	13000
Intestines	$k_{e,1}$	0.71	0.58	0.00013	3.2
Caeca	$k_{u,2}$	1300	1600	0.00027	1800
Caeca	$k_{e,2}$	0.0072	0.00076	$1.20.10^{-5}$	0.054
Cephalons	$k_{u,3}$	190	0.16	$1.60.10^{-5}$	2200
Cephalons	$k_{e,3}$	0.35	0.0089	$1.40.10^{-5}$	3
Remaining tissues	$k_{u,4}$	180	0.12	$1.50.10^{-5}$	2000
Remaining tissues	$k_{e,4}$	0.21	0.0041	$1.40.10^{-5}$	2.4
Intestines-Caeca	$k_{2,1}$	0.07	0.0013	$1.30.10^{-5}$	0.78
Intestines-Caeca	$k_{1,2}$	0.033	0.017	$1.50.10^{-5}$	0.11
Intestines-Cephalons	$k_{3,1}$	0.047	0.0025	$1.30.10^{-5}$	0.47
Intestines-Cephalons	$k_{1,3}$	0.49	0.034	$1.50.10^{-5}$	3.1
Intestines-tissues	$k_{4,1}$	0.057	0.0035	$1.30.10^{-5}$	0.54
Intestines-tissues	$k_{1,4}$	0.34	0.032	$1.50.10^{-5}$	2.3
Caeca-Cephalons	$k_{3,2}$	0.027	0.0037	$1.40.10^{-5}$	0.16
Caeca-Cephalons	$k_{2,3}$	0.41	0.01	$1.40.10^{-5}$	3.3
Caeca-tissues	$k_{4,2}$	0.047	0.022	$1.80.10^{-5}$	0.26
Caeca-tissues	$k_{2,4}$	0.3	0.013	$1.50.10^{-5}$	2.5
Cephalons-tissues	$k_{4,3}$	0.35	0.013	$1.40.10^{-5}$	2.7
Cephalons-tissues	$k_{3,4}$	0.18	0.0085	$1.40.10^{-5}$	1.5

Table 3: Medians of parameters estimated from the four-compartment TK model simultaneously fitted to each data set corresponding to the four identified organs of *Gammarus fossarum* exposed to dissolved Cd at 11.1 $\mu g.L^{-1}$ for 7 days, before being placed for 14 days under depuration conditions.

As a first step, parameter estimates need to be loaded within the R software: The corresponding tabular file, entitled `‘param4comp.txt‘`, with parameter estimates is provided as a Supplementary material on-line in the dedicated repository, available at `https://doi.org/10.5281/zenodo.6122201`. The script with the R command lines, entitled `‘script4comp.R‘`, is also downloadable from this repository.

Figures hereafter illustrate the final results of both simulation outputs, either based on the exact solution (Figure 4), or on the the numerical integration of the ODE system (Figure 5). These figures confirm the exact match between our generic solution of the multi-compartment TK model, with a numerical simulation for given parameter values.

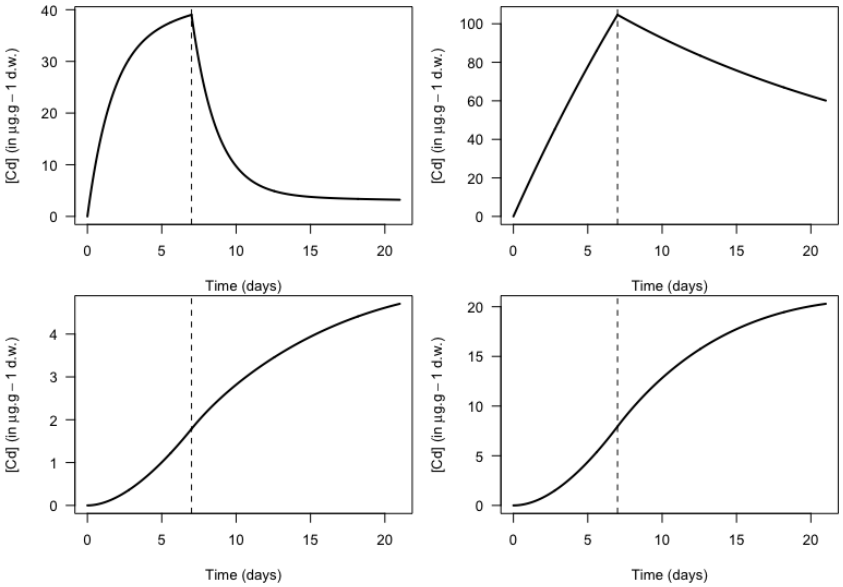

Figure 4: Simulation of the ODE matrix system from its exact solution given by equations 28a and 28a in all compartments. Parameter values are given in Table 3.

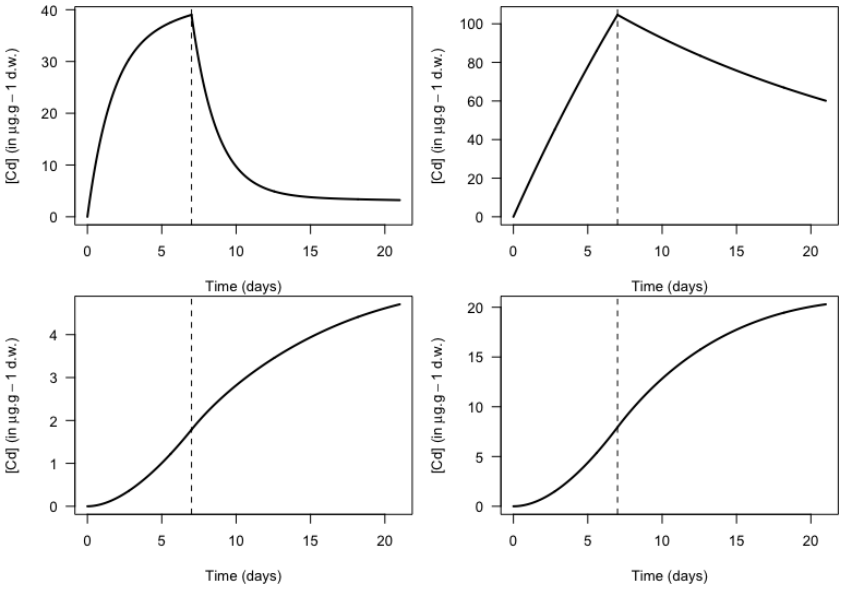

Figure 5: Simulation of the ODE matrix system given in equations 30 and 31, based on a numerical integration. Parameter values are given in Table 3.

5.2.2 Biologically-based connections between compartments

Based on the final results of [6], we provide below two sets of simulations both based on a four-compartment model but only considering biologically-founded compartment connections according to Figure 2 and the black solid arrows. This model assumes that only intestines are directly

connected to the external medium (here, water), and that it accounts for connections only between intestines and the three other organs. The first set of simulations corresponds to internal concentration measured within organs of *G. fossarum* when exposed to Cd at concentration 11.1 $\mu g.L^{-1}$(Figure 6). The second set of simulations is for *G. fossarum* exposed to Hg at concentration 0.27 $\mu g.L^{-1}$ (Figure 7). Parameter estimates are listed in Table 4 for both compounds. Tabular files with parameters as well as both R scripts are available in the Zenodo repository at `https://doi.org/10.5281/zenodo.6122201`.

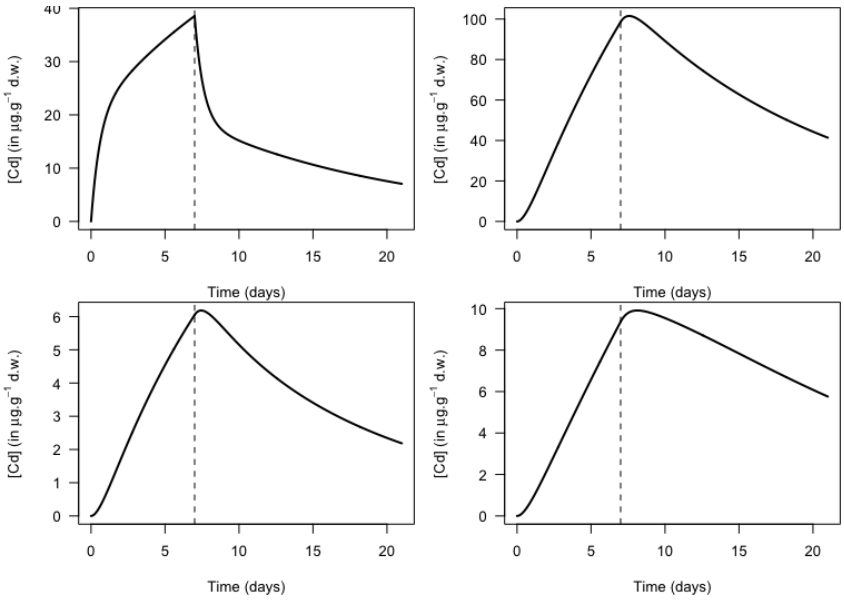

Figure 6: Simulation of the ODE matrix system given in equations 30 and 31, based on a numerical integration. Parameter values are given in Table 4.

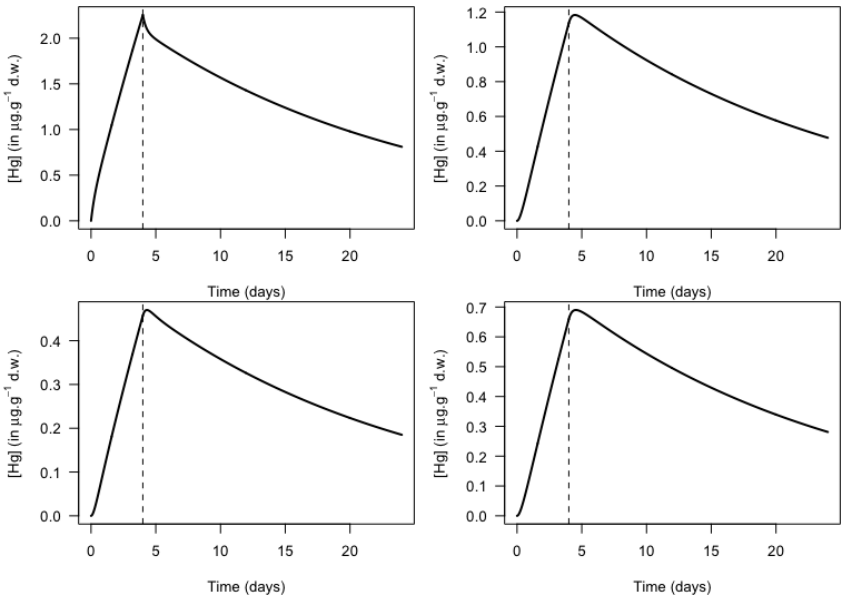

Figure 7: Simulation of the ODE matrix system given in equations 30 and 31, based on a numerical integration. Parameter values are given in Table 4.

Organ-Connection	Parameter	Median	$Q_{2.5\%}$	$Q_{97.5\%}$	Median	$Q_{2.5\%}$	$Q_{97.5\%}$
Intestines (uptake)	$k_{u,1}$	3342	2720	3707	4640	3890	5272
Intestines (elimination)	$k_{e,1}$	0.54	0.415	1.402	0.102	0.06	0.141
Intestines-Caeca	k_{21}	0.873	0.603	1.739	1.023	0.622	1.35
Caeca-Intestines	k_{12}	0.218	0.132	0.376	1.784	0.872	2.312
Intestines-Cephalons	k_{31}	0.059	0.034	0.124	0.515	0.269	0.653
Cephalons-Intestines	k_{13}	0.262	0.124	0.871	2.303	0.757	2.967
Intestines-Residues	k_{41}	0.069	0.049	0.126	0.552	0.405	0.714
Residues-Intestines	k_{14}	0.14	0.086	0.238	1.639	0.999	2.145
Intestines	σ_1	8.974	6.469	15.28	0.743	0.556	1.053
Caeca	σ_2	17.94	13.07	26.84	0.434	0.323	0.615
Cephalons	σ_3	1.223	0.863	1.818	0.076	0.056	0.113
Residues	σ_4	1.468	1.06	2.242	0.068	0.05	0.099

Table 4: Parameter estimates (expressed as medians and 95% uncertainty intervals) of the four-compartment model corresponding to black arrows in Figure 2 as provided by [6] in their Table S6. The first column stands for connected organs, either to water or to the other organs (see Figure 2, solid black arrows); the second column is for parameter names; the next three columns are for medians, lower and upper quantiles of parameter estimates when *G. fossarum* were exposed to Cd = 11.1 $\mu g.L^{-1}$; the last three columns are for medians, lower and upper quantiles of parameter estimates when *G. fossarum* were exposed to Hg = 0.27 $\mu g.L^{-1}$.

5.3 Five-compartment TK model