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The added value of Bayesian inference for estimating biotransformation rates of organic contaminants in aquatic invertebrates

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1 Abstract

2 Toxicokinetic (TK) models refer to the process of contaminant bioaccumulation as a
3 balance between rate of uptake from different sources (e.g. water or food), and rate of
4 elimination via different processes such as excretion, growth and/or biotransformation.
5 Biotransformation can considerably modify the fate of chemicals in an organism,
6 especially their bioavailability, residence time, and toxicity. Invertebrate models
7 generally neglect this process as they assume a low metabolic activity. However, some
8 species such as *Gammarus sp.* amphipods are able to metabolize a vast range of
9 organic compounds. Some recent TK models include biotransformation, but they prove
10 limited for estimating related parameters by giving negative values and/or large
11 uncertainties for biotransformation rate(s). Here we propose a generic TK model
12 accounting for biotransformation using a Bayesian framework for simultaneously
13 estimating the parameters. We illustrated the added value of our method by fitting this
14 generic TK model to 22 published datasets of several benthic invertebrate species
15 exposed to different chemicals. All parameters are estimated simultaneously for all
16 datasets and showed narrow estimates. Furthermore, the median model predictions
17 and their 95% credibility intervals showed that the model confidently fitted the data. In
18 most cases the uncertainties around biotransformation rate(s) were reduced in
19 comparison to the original studies. From a methodology standpoint, this paper reflects

1 that Bayesian inference has real added value for simultaneously estimating all TK
2 parameters for parent chemicals and their metabolite(s) based on all available data,
3 while accounting for different types of data and the correlation between parameters.
4 Bayesian inference was able to overcome the limits of previous methods, since no
5 parameters were fixed and no irrelevant negative values were obtained. Moreover, the
6 95% credibility intervals around model predictions, which are core uncertainties for
7 Environmental Risk Assessment, were easily acquired.

8
9 **Keywords:** Invertebrate – Biotransformation – Bayesian inference – Toxicokinetic
10 model – Bioaccumulation

11
12 **Highlights:**

- 13 - A Bayesian framework was developed for a TK model considering biotransformation
14 - It was applied to *Chironomus*, *Gammarus*, *Hyalella*, *Nereis* and *Lumbriculus* genus
15 - It was applied to various chemicals (PAHs, drugs and plant protection products)
16 - In ERA, quantification of uncertainties around model predictions are crucial
17 - For metabolites, uncertainties around parameters were reduced

1. Introduction

2 Biotransformation of chemicals can significantly modify the fate of chemicals in
3 an organism, especially their bioavailability, toxicity, and residence time in the
4 environment (e.g. water, sediment) and/or in an organism (Livingstone 1998). A
5 chemical compound has to enter the organism to have effect, so biotransformation
6 means that it accumulates less target chemical (parent compound) compared to
7 untransformed chemical. Firm knowledge of the major quantitative and qualitative
8 resemblances and dissimilarities in biotransformation pathways between species is a
9 critically-needed foundation for designing toxicity tests, developing biomarkers,
10 modeling chemical fate in ecosystems, and understanding the selective part of
11 biotransformation in animal ecology and evolution (Livingstone 1994).

12 Directive 2008/105/EC, the Environmental Quality Standards Directive, defines
13 the good chemical status to be achieved by all Member States from Europe and,
14 together with Water Framework Directive 2000/60/EC, lays down the legal basis for
15 monitoring of priority substances in sediment and biota. For several substances of the
16 listed priority substances and certain other pollutants included in the Directive, the
17 establishment of Community level Environmental Quality Standards (EQS) is limited to
18 parent compound concentrations in biota, and fails to consider metabolites and their
19 potential toxic effects on the organism.

20 Substantial progress has been made in measuring metabolites and estimating
21 biotransformation rates for organic chemicals in fish (Arnot *et al.* 2008a, Arnot *et al.*
22 2008b, Nichols *et al.* 2007) and, to a lesser extent, in other aquatic organisms (Katagi,
23 2010). Biotransformation by aquatic invertebrates has often been supposed to be
24 limited compared to fish (Morrison *et al.* 1996), as invertebrates are thought to have
25 low metabolic activity. Nonetheless, researches conducted during the past decade
26 have shown that several invertebrate species are able to metabolize various chemicals
27 (Ashauer *et al.* 2012, Fu *et al.* 2018, Jeon *et al.* 2013, Miller *et al.* 2017, Rösch *et al.*
28 2016). For example, metabolites were quantitatively measured in *Daphnia magna* and
29 *Gammarus fossarum* exposed to drugs and plant protection products (Jeon *et al.*
30 2013).

31 In Environmental Risk Assessment (ERA), models based on toxicokinetic (TK)
32 are commonly used to predict contaminant concentrations in organisms from those to
33 which they are exposed in their environment (EPA 2006). For aquatic invertebrates,
34 compartmental models are often used, where the organism is considered as a single
35 compartment. In TK models, biotransformation could be considered as part of total
36 elimination (MacKay and Fraser 2000): the biotransformation rate points out how much
37 biotransformation contributes to the reduction of parent compound bioaccumulation.
38 Van der Linde *et al.* (2001) contended that including the biotransformation term in TK
39 models could correct the overestimation of internal concentrations, even in the
40 absence of experimental data for metabolites. They proposed a “difference method” to
41 estimate biotransformation rate by considering it as the difference between the global
42 elimination rate and the sum of the rates for other dissipation processes (growth
43 dilution, excretion). Arnot *et al.* (2008b) further developed a similar approach for
44 nonionic organic chemicals, where biotransformation rate constants were calculated
45 as the difference between a measured bioconcentration factor or elimination rate
46 constant, and a model-derived bioconcentration factor or elimination rate constant
47 estimated assuming no biotransformation. Chen and Kuo (2018) also estimated
48 biotransformation rates from whole-body biotransformation half-lives, which were

1 estimated on the basis of free dissolved chemical fraction. However, these approaches
2 present drawbacks, chiefly that they lead to large uncertainties around
3 biotransformation rates and in some cases have even yielded negative values.

4 Estimating biotransformation rates in invertebrate species by fitting a TK model
5 to experimental data gained further appeal with a study by Ashauer *et al.* (2012), who
6 used bioaccumulation kinetics of 11 xenobiotics and their metabolites in *Gammarus*
7 *pulex*. They constrained the parameters to positive values and estimated them by
8 least-squares minimization without weighting data. Apart from two compounds where
9 the uptake rate from water needed to be kept fixed, all parameters were estimated
10 simultaneously. Also, the elimination rate of the parent compound reached its lower
11 boundary zero for five chemicals, large confidence intervals were obtained for the
12 uptake and elimination rates of three compounds, and uncertainties around mean
13 predictions were missing. More reliable risk assessment hinges on properly estimating
14 parameters with their uncertainties and considering their between-parameters
15 correlation. Finally, Kuo and Chen (2016) proposed a generic theoretical framework
16 allowing to estimate biotransformation rates from kinetic observations of the parent
17 contaminant and its metabolites. Their framework was applied to two polychaetes
18 (*Nereis diversicolor* and *Tubifex tubifex*) and the oligochaete *Lumbriculus variegatus*
19 using data for two chemicals, i.e. pyrene and 2,4,6-trinitrotoluene, and parameters
20 were estimated by least-squares minimization of errors. The parent-compound
21 parameters were either fixed to the values reported in the original studies, or estimated
22 with the best fit obtained, and then fixed to estimate only two parameters, i.e.
23 metabolite biotransformation rate and metabolite elimination rate. Their method could
24 be readily incorporated into standard experimental protocols. However, as all
25 illustrations in their research dedicated to worm species, further validations across
26 diverse biological species would be suitable, especially for the main species studied in
27 bioaccumulation studies (fish and invertebrates). Furthermore, they only considered
28 the simplest case of constant exposure level and they applied the model only to the
29 uptake phase, neglecting the elimination phase. A step forward would be to revisit the
30 existing biotransformation literature with their approach on well-studied organic
31 contaminants such as polycyclic aromatic hydrocarbons (PAHs) and on chemicals of
32 growing environmental concern.

33 As biotransformation depends on biological pathways of the organism and the
34 mode of action of the chemical, TK models must adapt to the specie and to the
35 chemical considered to include the biotransformation process. Thus, this study
36 introduces a unified Bayesian inference framework to estimate parameters of TK
37 models accounting for biotransformation processes. This approach is based on
38 simultaneously estimating all kinetic parameters from accumulation and depuration
39 data for the parent compound and its metabolite(s). In total, 22 experimental datasets
40 from the literature encompassing six invertebrate aquatic species commonly used in
41 ERA (i.e. *Gammarus pulex*, *Gammarus setosus*, *Chironomus tentans*, *Hyalella azteca*
42 *Nereis diversicolor*, and *Lumbriculus variegatus*) exposed to 17 chemicals from water
43 and/or diet. TK models were fitted on each couple chemical-species. We recommend
44 the Bayesian approach because it allows to estimate TK parameters without
45 constraining or fixing the values, while accounting for the correlations between
46 parameters. This approach also provides an accurate assessment of the uncertainty
47 around parameters estimates and model predictions.

2. Material and methods

2.1. A generic TK model accounting for biotransformation

A generic first-order TK model can be written as follows (Eqs. (1) and (2), Ratier *et al.* (2019)):

$$\left\{ \begin{array}{l} \frac{dC(t)}{dt} = \sum_{i=1}^n (k_i \times C_i(t)) - \sum_{j=1}^p (k_j \times C(t)) \text{ for } 0 \leq t \leq t_c \end{array} \right. \quad (1)$$

$$\left\{ \begin{array}{l} \frac{dC(t)}{dt} = - \sum_{j=1}^p (k_j \times C(t)) \text{ for } t > t_c \end{array} \right. \quad (2)$$

where $C(t)$ is contaminant concentration at time t (days) in the whole organism ($\text{ng g}_{\text{org}}^{-1}$ or $\text{nmol kg}_{\text{org}}^{-1}$ where mass is expressed in wet weight (ww)), k_i is uptake rate from the contamination source i (day^{-1}), $C_i(t)$ is contaminant concentration (like $C(t)$, i.e. in ng ml^{-1} if $C(t)$ in ng g^{-1}) in the contamination source i at time t (day), n is number of exposure sources, k_j is the rate for the elimination process j (day^{-1}), p is number of elimination routes and the parameter t_c corresponds to the duration of the accumulation phase (days). In classical bioaccumulation experiments, this accumulation phase (Eq. (1)) is generally followed by a depuration phase where organisms are placed in clean (contaminant-free) medium. Eq. (2) thus corresponds to this depuration phase, in which only elimination processes take place.

Biotransformation is included as an elimination route (k_j) for the parent compound, and the specific biotransformation rate is noted k_m . Thus, Eq. (1) and (2) could be rewritten for the parent compound as follows (Eqs. (3) and (4)), by distinguishing biotransformation from other elimination processes:

$$\left\{ \begin{array}{l} \frac{dC(t)}{dt} = \sum_{i=1}^n (k_i \times C_i(t)) - \sum_{d=1}^q (k_d \times C(t)) - \sum_{x=1}^z (k_{m,x} \times C(t)) \text{ for } 0 \leq t \leq t_c \end{array} \right. \quad (3)$$

$$\left\{ \begin{array}{l} \frac{dC(t)}{dt} = - \sum_{d=1}^q (k_d \times C(t)) - \sum_{x=1}^z (k_{m,x} \times C(t)) \text{ for } t > t_c \end{array} \right. \quad (4)$$

where k_d is the rate associated to elimination process d other than biotransformation, q is the number of elimination process other than biotransformation, $k_{m,x}$ is the biotransformation rate of a metabolite x (day^{-1}) and z is number of metabolites.

These equations depicting the bioaccumulation and depuration of the parent compound should then be coupled with the equation concerning the corresponding metabolite(s) (Eq. (5)):

$$\frac{dC_{\text{met},x}(t)}{dt} = \sum_{x=1}^z (k_{m,x} \times C(t)) - \sum_{x=1}^z (k_{\text{emet},x} \times C_{\text{met},x}(t)) \quad (5)$$

where $C_{\text{met},x}(t)$ is the concentration of metabolite x at time t (days) in the whole organism (ng g^{-1} or nmol kg^{-1} ww) and $k_{\text{emet},x}$ is elimination rate of metabolite x (day^{-1}).

1 The model is adapted to the contamination-source data, i.e. water or sediment.
2 We supposed that (i) metabolites come exclusively from the parent compound, (ii) they
3 are not reuptaken, (iii) they are eliminable and (iv) the concentration of parent
4 compound is constant over time in media. Analytical solutions are provided for the
5 parent compound and for its metabolites for both the uptake and elimination phases
6 (Annex S1).

7 **2.2. Datasets from the literature and associated inference methods**

9 In literature, we found seven studies reporting biotransformation kinetics in
10 aquatic invertebrates of 22 parent compounds, including PAHs, drugs and plant
11 protection products (Giessing *et al.* 2003, Schuler *et al.* 2003, Mäenpää *et al.* 2009,
12 Ashauer *et al.* 2012, Carrasco-Navarro *et al.* 2015, Miller *et al.* 2017, Fu *et al.* 2018).
13 In each case, the generic model (Eqs (3-4) and (5-6)) was adapted to these
14 biotransformation TK data. For all the datasets, only one exposure route was
15 considered, i.e. by sediment or by water according to the study (Table 1). A single
16 metabolite was often followed (Giessing *et al.* 2003, Schuler *et al.* 2003, Mäenpää *et*
17 *al.* 2009, Ashauer *et al.* 2012, Carrasco-Navarro *et al.* 2015, Miller *et al.* 2017), but two
18 or three metabolites were followed during the tests in a few studies (Ashauer *et al.*
19 2012, Miller *et al.* 2017, Fu *et al.* 2018) (Table 1). Only experimental data were reported
20 in one study (Giessing *et al.* 2003) whereas the others estimated parameters of the TK
21 model for parent compounds, and, less often, for the metabolite (Ashauer *et al.* 2012,
22 Carrasco-Navarro *et al.* 2015, Fu *et al.* 2018).

23 Briefly, Miller *et al.* (2017) exposed for two days *Gammarus pulex* to propranolol
24 and diazepam spiked water. The uptake rate constant (k_w) and elimination rate
25 constant (k_e) were obtained using a curve fitting algorithm, using either the
26 simultaneous or sequential modelling methods.

27 For 24 hours, Ashauer *et al.* (2012) exposed *Gammarus pulex* to water spiked
28 with 2,4-dichloroaniline, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 4-nitrobenzyl-
29 chloride, aldicarb, carbaryl, carbofuran, chlorpyrifos, malathion, pentachlorophenol
30 and seanine. They constrained the parameters to positive values. They were obtained
31 without weighting of data by least-squares minimization (Levenberg–Marquardt
32 algorithm). Asymptotic standard errors of parameters were obtained from the
33 covariance matrix and by calculation of confidence intervals. Except for 4-nitro-benzyl-
34 chloride and carbaryl where the uptake rate from water (k_w) needed to be kept fixed at
35 the value from a previous study (Ashauer *et al.* 2010) in order to reach a good fit, all
36 parameters, for a given compound, were estimated simultaneously.

37 Carrasco-Navarro *et al.* (2015) exposed for four days *Gammarus setosus* to
38 pyrene spiked water. In order to estimate the initial values of the parameters before
39 running the inference process, they selected a model to fit the data into the Sigma Plot
40 library. Then, a TK model was used to fit experimental data of total body burden, the
41 parent pyrene concentrations, and the sum of all metabolites. The TK parameters were
42 estimated by a least-squares fit.

43 Schuler *et al.* (2003) exposed *Lumbriculus variegatus* and *Chironomus tentans*
44 to benzo(a)pyrene spiked sediment for 10 and 3 days, respectively. TK parameters
45 were estimated by a two-step procedure: first, assuming that elimination can be
46 neglected during the initial segment of the uptake phase, uptake rate from sediment
47 (k_s) was obtained from the uptake phase. Second, an iterative least-squares method
48 was used to estimate the other parameters when the models were fitted to data.

1 Mäenpää *et al.* (2009) exposed *Lumbriculus variegatus* to pyrene spiked water
2 for 15 days. Their experiment was limited to the uptake phase. The TK model was fitted
3 to the pyrene concentration in the test organisms, where only parent compound
4 parameters were estimated by nonlinear regression. Relative parameters to metabolite
5 were not estimated since only the experimental metabolite concentrations in organisms
6 were given.

7 Giessing *et al.* (2003) exposed *Nereis diversicolor* to pyrene-spiked sediment
8 for 9 days. Only the experimental concentrations of pyrene and its metabolite in
9 organisms were reported. Parameters were not estimated.

10 Fu *et al.* (2018) exposed for 24 hours *Hyalella azteca* and *Gammarus pulex* to
11 water spiked with azoxystrobin and prochloraz. They made a distinction among the
12 parent compounds, the sum of all measured primary metabolites which come directly
13 from the parent compound, and of the total of all secondary metabolites. To estimate
14 the parameters, they contrast two approaches: stepwise fitting (by fixing the uptake
15 rate to estimate the other parameters, where a greater significance was attributed to
16 the uptake rate from water, k_w) and simultaneously (constrained to positive values).
17 Comparable results were achieved for both approaches.

18 In summary, we selected these 22 datasets to consider several invertebrates
19 species (polychaetes, oligochaetes, diptera and amphipods) encompassing different
20 ways of life (epibenthic and endobenthic), exposed via spiked water or sediment to
21 various chemicals (17 compounds including two PAHs, two drugs, 10 plant protection
22 products and three precursors in their synthesis) with different properties (low to high
23 hydrophobicity, $\log K_{ow}$ between 1.13 and 6.13), with variable numbers of metabolites,
24 and contrasting approaches to estimate the parameters.

1 **Table 1.** Summary of the chosen literature data where biotransformation kinetics in
 2 invertebrates are reported.

	Species	Reference	Chemicals	Exposure route	TK parameters availability
	<i>Gammarus</i> sp.	Miller <i>et al.</i> , 2017	Propranolol	water	Incomplete (k_m and k_{emet} were missing)
		Fu <i>et al.</i> , 2018*	Azoxystrobin Prochloraz	water	All
		Ashauer <i>et al.</i> , 2012	Aldicarb Carbaryl Carbofuran Malathion Pentachlorophenol	water	All
		Carrasco-Navarro <i>et al.</i> , 2015	Pyrene	water	All
1 metabolite	<i>Chironomus tentans</i>	Schuler <i>et al.</i> , 2003	Benzo(a)pyrene	sediment	Incomplete (k_{emet} is missing)
	<i>Lumbriculus variegatus</i>	Schuler <i>et al.</i> , 2003	Benzo(a)pyrene	sediment	Incomplete (k_{emet} is missing)
		Mäenpää <i>et al.</i> , 2009	Pyrene	water	Incomplete (k_m and k_{emet} are missing)
	<i>Nereis diversicolor</i>	Giessing <i>et al.</i> , 2003	Pyrene	sediment	None
	<i>Hyalella azteca</i>	Fu <i>et al.</i> , 2018*	Azoxystrobin Prochloraz	water	All
2 metabolites	<i>Gammarus pulex</i>	Ashauer <i>et al.</i> , 2012	Chlorpyrifos 2,4-dichloroaniline 2,4-dichlorophenol 2,4,5-trichlorophenol	water	All
3 metabolites	<i>Gammarus pulex</i>	Ashauer <i>et al.</i> , 2012	Seanine 4-nitrobenzyl-chloride	water	All (k_w fixed for 4-nitrobenzyl-chloride)
		Miller <i>et al.</i> , 2017	Diazepam	water	Incomplete (k_m was missing)

3 *: The second metabolite originated from first metabolites were not considered.

2.3. Bayesian inference framework

2.3.1. The TK model(s)

Considering only one exposure pathway (water or sediment depending on the data) and in accordance with the number of metabolites, Eqs. (3) and (5) can thus be adapted for a parent compound and its metabolites (Eq. (6) for the uptake phase and Eq. (7) for the elimination phase), for each species/chemical couple applied separately:

$$\begin{cases}
 \frac{dC(t)}{dt} = k_w \times C_w - (k_e + k_{m,1} + \dots + k_{m,z}) \times C(t) \\
 \frac{dC_{met,1}(t)}{dt} = k_{m,1} \times C(t) - k_{emet,1} \times C_{met,1}(t) \\
 \cdot \\
 \cdot \\
 \cdot \\
 \frac{dC_{met,z}(t)}{dt} = k_{m,z} \times C(t) - k_{emet,z} \times C_{met,z}(t)
 \end{cases}
 \quad \text{for } 0 \leq t \leq t_c \quad (6)$$

$$\begin{cases}
 \frac{dC(t)}{dt} = - (k_e + k_{m,1} + \dots + k_{m,z}) \times C(t) \\
 \frac{dC_{met,1}(t)}{dt} = k_{m,1} \times C(t) - k_{emet,1} \times C_{met,1}(t) \\
 \cdot \\
 \cdot \\
 \cdot \\
 \frac{dC_{met,z}(t)}{dt} = k_{m,z} \times C(t) - k_{emet,z} \times C_{met,z}(t)
 \end{cases}
 \quad \text{for } t > t_c \quad (7)$$

where C_w (or C_s) is parent-compound concentration in water (or sediment) ($\mu\text{mol L}^{-1}$, nmol L^{-1} , $\mu\text{g mL}^{-1}$, $\mu\text{g L}^{-1}$ or $\text{ng g}_{\text{sed}}^{-1}$), k_w (or k_s) is the uptake rate from water (or from sediment) of the parent compound (day^{-1}), $k_{m,x}$ is biotransformation rate of the parent compound to the metabolite, $C_{met,x}$ (for $x = 1 \dots z$) is concentration of the metabolite x and $k_{emet,x}$ is elimination rate of metabolite x . In the datasets used, we have data for one, two or three metabolites ($z = 1, 2$ or 3).

2.3.2 Stochasticity

For the contaminant concentration accumulating in the organism (parent or metabolite), we supposed a Gaussian distribution (Eq. (8) or (9)):

$$C_{obs}(t) \sim N(C(t), \sigma) \quad (8)$$

$$C_{obs,met,x}(t) \sim N(C_{met,x}(t), \sigma_{met,x}) \quad (9)$$

where N stands for normal distribution, $C_{obs}(t)$ corresponds to the chemical concentration in the organism at time t measured during the experiments, $C_{obs,met,x}(t)$ is concentration of metabolite x in the organism at time t measured during the experiments, $C(t)$ is concentration of contaminant in the organism at time t predicted by the model (Eqs. (6) and (7)), $C_{met,x}(t)$ is concentration of metabolite x in the organism at time t predicted by the model (Eqs. (6) and (7)), σ is the standard deviation of the concentration contaminant in the organism, and $\sigma_{met,x}$ is the standard deviation of the concentration of metabolite x in the organism.

In Figure S1, the generic directed acyclic graph (DAG) for an organism exposed to a parent compound and its metabolite(s) is given.

2.3.3. Definition of priors

The prior distributions of the parameters are summarized in Table S1. They were defined as non-informative uniform decimal logarithm ($-5, 2$) distributions, except where previous studies had already estimated a parameter or reported available data: in this case, we can assume a normal prior distribution with the estimated mean value and the precision with a standard deviation (e.g. in the study of Ashauer et al., (2012)), in order to account for the possible differences in experimental conditions, at least twice the value estimated. For more details, when no previous data were available (other than the original study), a uniform distribution was assumed because general prior information was available: parameters could not be negative (for a biological meaning) and it is often noticed that TK parameters are ranging between 0.00001 and 100. Thus, a uniform decimal logarithm ($-5, 2$) distribution was defined for most datasets. However, for some datasets, this distribution was not sufficient (i.e. when parameter estimated was closed to the limit, i.e. 0.00001 or 100). In this particular case, the prior distribution was enlarged until the estimated parameter was not too closed to the limit (Table S1). This was the case for malathion, 2,4,5-trichloro-phenol and 2,4-dichloro-phenol datasets (for k_e), chlorpyrifos, 2,4,5-trichloro-phenol and 2,4-dichloro-phenol (for k_w) and pyrene for *G. setosus* and *Nereis diversicolor* (all parameters). Priors were defined on the decimal logarithm scale in order to give the same probability to low or high values. As a non-informative (0.001, 0.001) Gamma prior on the precision is usually used for variance parameters (Lambert *et al.* 2005, Richards and Chaloupka 2009), we also assumed that prior distribution.

2.3.4. MCMC simulations

JAGS and R software were used to perform inference computation (Plummer 2019, R Core Team 2020). Bayesian inference via Markov Chain Monte Carlo (MCMC) sampling was used to fit the models to data. For each dataset, in order to set the required thinning and number of iterations to reach an accurate estimation of each parameter, we ran a short sampling phase (a burn-in phase of 20,000 iterations, then 5,000 iterations) for three chains using the Raftery and Lewis (1992) method (Table S1). Furthermore, we used visual inspection as well as the Gelman criterion (Gelman, 1995) or Geweke criteria (Geweke 1989) to monitor the convergence of the three chains. The R codes are available in supporting information (Annex S2). At the end, we kept between 500 and the 14,285 last iterations for each chain according to the data, meaning that the joint posterior distribution ranged between 1,500 to 42,857 sets of parameters, all simultaneously estimated and thus taking into account between-parameter correlation.

2.3.5. Parameter posterior distributions and model predictions

From the joint posterior distribution, we deduced the marginal posterior distribution for each parameter, which can be summarized by the mean (or the median) and by the 95% credibility interval (quantiles of the distributions, in brackets). The accuracy of parameter estimation can be visualized by comparing prior and posterior distributions: the data bring enough information to precisely estimate parameters when a narrower posterior distribution is obtained than prior distribution. To check the relevance of model predictions, we plotted each dataset as the observed data superimposed over the mean model prediction (using the mean value obtained for each parameter) and the 95% credibility interval (CI). To obtain the 95% CI, we ran a model simulation using the stochastic part (Eq. 8 and 9) with all the joint posterior distribution and then

1 calculated the quantiles at 2.5% and 97.5% for each time step. Posterior Predictive
2 Checks (PPC) were also graphed in order to illustrate the goodness-of-fit. The PPC
3 plotted the observed data against the corresponding predicted value from the fitted
4 model as a 95%-credible interval. If the fit is correct, then 95% of the observed values
5 are expected to fall inside credible intervals. Each dot corresponds to one observation
6 from the dataset, and the corresponding observed value can be read on the x-axis,
7 while the y-axis provides the value predicted by the fitted model as well as the 95%
8 credible interval. All these criteria came from the EFSA recommendations on TKTD
9 models (Ockleford *et al.*, 2018).

10

11 A guidance is provided in supporting information (Annex S3) that gives the steps
12 for applying this framework to an experimental dataset.

3. Results

3.1. Parameter estimates and model predictions

The inference process converged without constraining or fixing any parameter for all datasets. For each parameter, narrow posterior distributions were obtained, meaning that data provided enough information to accurately estimate parameters (Fig. S2). The mean value and 95% CI (in bracket) for each parameter and each species-contaminant pair are reported in Table S1.

The between-species differences in metabolic capacity were reported by the original studies. In the benzo(a)pyrene spiked sediment experiments (Schuler *et al.*, 2003), chironomids had a better biotransformation capacity than *L. variegatus* ($k_{m,1} = 1.66$ and $k_{m,2} = 0.038$ d⁻¹, respectively), as the authors had already denoted. In the work of Fu *et al.* (2018), comparable biotransformation rates were obtained for gammarid and hyalellid species exposed to prochloraz and azoxystrobin. Furthermore, for pyrene, $k_{m,1}$ was higher in *G. setosus* and *L. variegatus* than *N. diversicolor* ($k_{m,1} = 1.04$, 1.54 and 0.0500 d⁻¹, respectively), possibly due to the exposure route (sediment exposure for *N. diversicolor*) accordingly to the authors.

The highest k_m values were obtained for organochlorine chemicals such as in *G. pulex* exposed to 2,4-dichlorophenol ($k_{m,1} = 39.5$ [13.0-93.5] d⁻¹), 2,4,5-trichlorophenol ($k_{m,1} = 38.2$ [3.11-93.8] d⁻¹) and 4-nitrobenzyl-chloride ($k_{m,1} = 42.3$ [30.8-58.5] and $k_{m,2} = 43.9$ [24.6-76.5] d⁻¹). The lowest values were obtained for PAHs, such as *L. variegatus* exposed to benzo(a)pyrene ($k_{m,1} = 0.0380$ [0.0270-0.0480] d⁻¹) and *N. diversicolor* exposed to pyrene ($k_{m,1} = 0.0500$ [0.0310-0.0730] d⁻¹).

Model predictions generally showed good fit with experimental data for the parent compound but also for its metabolite(s) (Fig. 1 and S3), whatever the number of metabolites (from one to three, Fig. 1-a and 1-e), and even when only accumulation data were available, as for *L. variegatus* and *N. diversicolor* exposed to pyrene (Giessing *et al.* 2003, Mäenpää *et al.* 2009, Fig. 1-b and -c). However, model predictions showed an unsatisfying fit in the elimination phase of the parent compound for about half of the chemicals studied (propranolol, azoxystrobin (Fig. 1-d), chlorpyrifos, carbaryl, 2,4-dichlorophenol, malathion, pentachlorophenol, 4-nitrobenzyl-chloride (Fig. 1-e), 2,4,5-trichlorophenol and seanine) and consequently underestimated the elimination rate. This result was also clearly visible on the respective PPCs (Fig. S4).

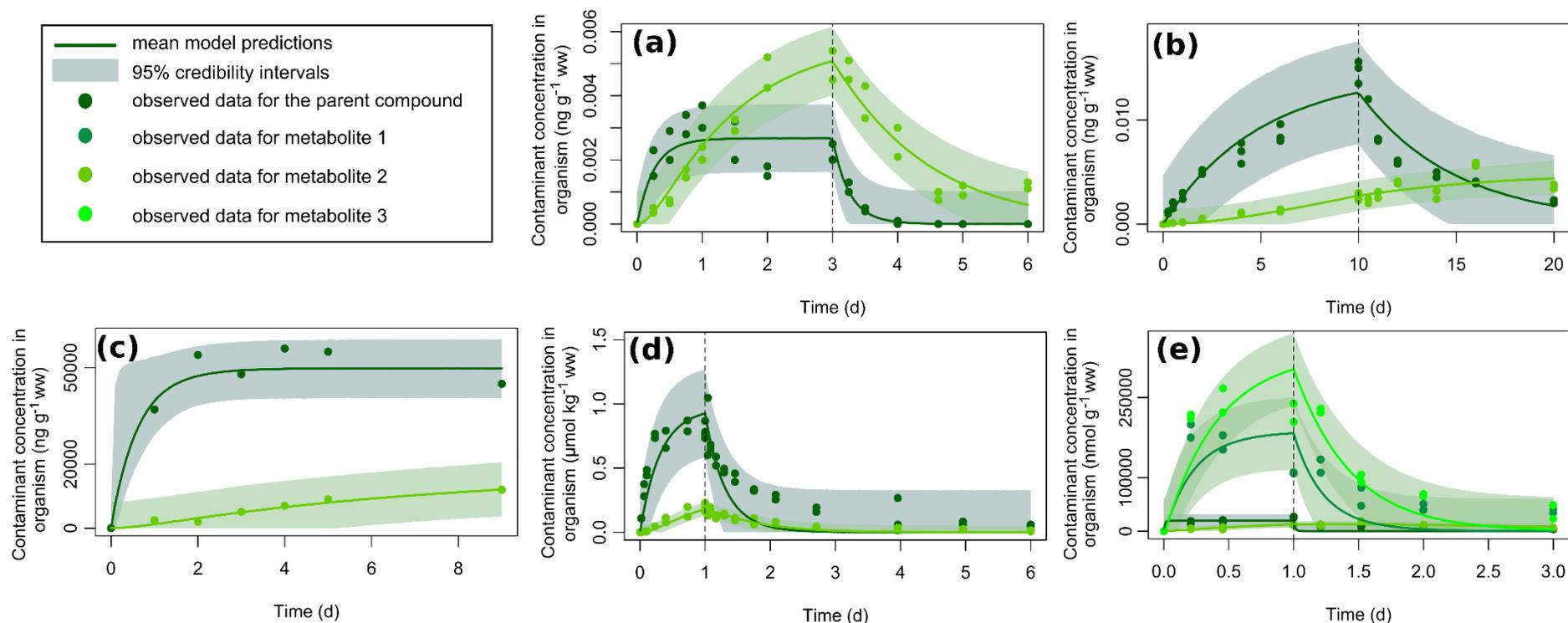
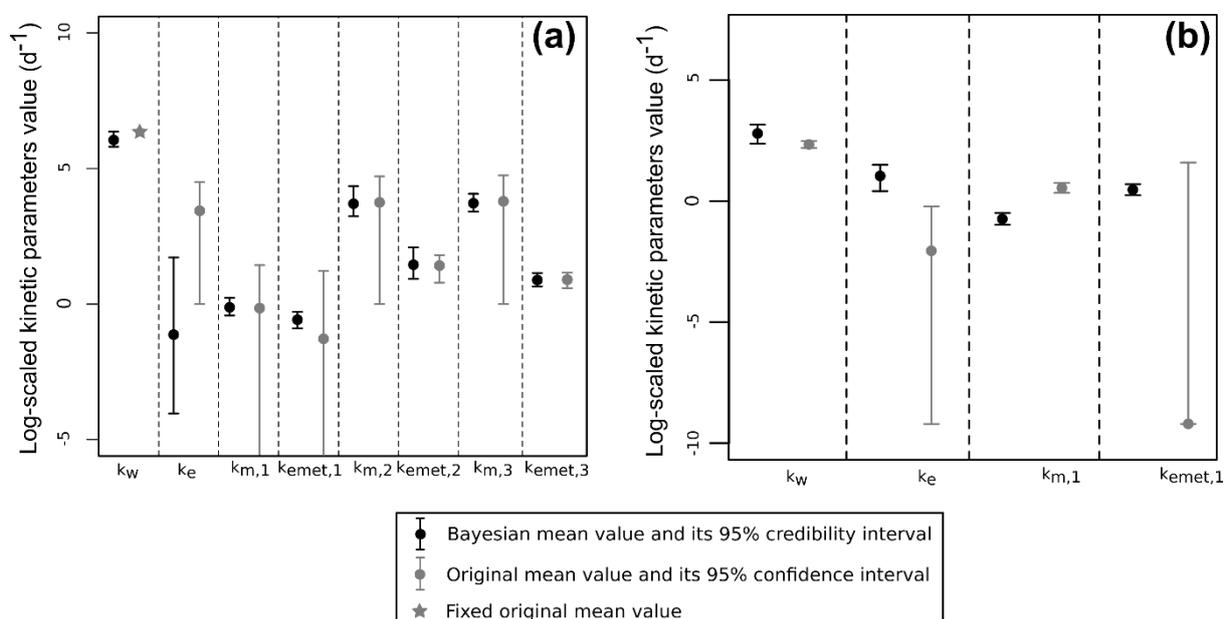


Figure 1. Examples of observed data (dots) and model predictions (plain line and gray area) for each parent compound and its metabolite(s) (Eqs. 6 to 9) plotted against time (days): (a) *C. tentans* exposed to benzo(a)pyrene (data from Schuler *et al.*, 2003), (b) *L. variegatus* exposed to benzo(a)pyrene (data from Schuler *et al.*, 2003), (c) *N. diversicolor* exposed to Pyrene (data from Giessing *et al.*, 2003), (d) *H. azteca* exposed to Azoxystrobin (data from Fu *et al.*, 2018) and (e) *G. pulex* exposed to 4-nitrobenzyl-chloride (data from Ashauer *et al.*, 2012). The uptake and depuration phases are separated with the dashed vertical line.

1 The 95% CI were added to the fits of predictions: the credibility band is relatively
 2 narrow, reflecting a relatively low variability, even if there were some cases where
 3 uncertainties around model predictions were wider (chlorpyrifos, malathion, 2,4,5-
 4 trichlorophenol, propranolol, diazepam and pyrene).

6 **3.2. Comparison of parameter values and uncertainties between original** 7 **estimations and those obtained by Bayesian inference**

8 When possible, a comparison was made between parameters values and their
 9 uncertainties obtained with our Bayesian inference method and the values and
 10 uncertainties given in original publications. In particular, two studies (Ashauer *et al.*
 11 2012, Fu *et al.* 2018) out of the seven chosen (i.e. 15 of the 22 datasets) had reported
 12 the mean estimated values for each parameter and their corresponding 95%
 13 confidence intervals. Here we selected those with the most relevant results (Fig. 2).



16 **Figure 2.** Log-scaled parameters (d^{-1}) estimated by Bayesian inference (black) and by
 17 the original method (grey) for (a) *G. pulex* exposed to 4-nitrobenzyl-chloride (Ashauer
 18 *et al.*, 2012), and (b) *H. Azteca* exposed to azoxystrobin (Fu *et al.*, 2018). Dots
 19 represent mean values, stars represent fixed mean values of parameter and error bars
 20 report 95% credibility intervals (Bayesian inference) or confidence intervals (original
 21 studies).
 22

23
 24 When the uptake rates were fixed (carbaryl and 4-nitro-benzylchloride, Ashauer *et*
 25 *al.*, 2012, Fig. 2-a), the uncertainty of the parameters correlated to those that had been
 26 fixed (k_w) was necessarily underestimated in the original studies, which is why CI
 27 around correlated parameters were wider with the Bayesian inference method. Indeed,
 28 for the carbaryl dataset (data not shown), k_w and k_e are highly correlated ($r^2 = 0.87$)
 29 and the uncertainties for k_e were larger than in the original study. In the case of 4-nitro-
 30 benzylchloride (Fig 2-a), k_w and $k_{m,2}$ are highly correlated ($r^2 = 0.81$) and the
 31 uncertainties for $k_{m,2}$ were larger than in the original study.

32 Otherwise, the mean k_m values were similar between our Bayesian inference and
 33 the original methods in 19 out of 27 cases. For the 2,4,5-trichlorophenol, prochloraz

1 and azoxystrobin (Fig. 2-b) datasets, the range of k_m values was outside the confidence
2 interval of the original study. In addition, estimated values of parent-compound
3 parameters were sometimes outside of the confidence interval obtained in the original
4 study (chlorpyrifos, malathion, diazepam, prochloraz and azoxystrobin). As illustrated
5 in Figure 2-b for *H. azteca* exposed to azoxystrobin, the mean estimated k_m was 0.47
6 [0.37-0.60] d⁻¹ with the Bayesian inference method against 1.8 [1.4-2.1] d⁻¹ with the
7 original method. The two uncertainty intervals did not overlap (Fig. 2b). Moreover, the
8 mean estimated elimination rate was 2.88 [1.57-4.66] d⁻¹ in Bayesian inference against
9 0.13 [0.0001-0.8] d⁻¹ with the original method. As observed for k_m , the two k_e
10 uncertainty intervals did not overlap (Fig. 2b).

1 4. Discussion

2 **4.1. Computational performance**

3 Here we propose using a Bayesian inference method to estimate the parameters of
4 a generic TK model accounting for biotransformation. In previous work, we set out the
5 various advantages of using Bayesian inference for modeling TK in freshwater benthic
6 invertebrates (Ratier *et al.*, 2019). In this paper, this approach was tested on data
7 available for different species exposed to different contaminants, with either only
8 bioaccumulation data, either bioaccumulation and depuration data, and with one or
9 more metabolites.

10 There are three main limitations to estimating the parameters accounting for
11 biotransformation in previous studies: (i) the need in some instances to fix one or more
12 parameter values in order to estimate the others, (ii) outputs providing sometimes
13 negative values for some parameters (e.g. k_m , Arnot *et al.*, 2008a, 2008b) and (iii)
14 missing uncertainties around mean model predictions. Bayesian inference overcomes
15 these limits as we did not fix any parameters, we did not obtain negative values for k_m ,
16 and we were able to provide the 95% credibility intervals around model predictions in
17 addition to correlations between parameters.

18 Uncertainties within environmental risk assessments need to be properly
19 characterized in order to use risk estimates as a sound basis for informing risk
20 management actions. In our modeling framework, we added the 95% credibility bands
21 around model predictions. However, we did find large uncertainties around model
22 predictions for some chemicals. Having larger uncertainties around parameters does
23 not mean that the Bayesian inference process is less efficient than the one used in the
24 original study. These large uncertainties could mainly be explained by incomplete
25 experimental data. For example, it is possible that not all the metabolites were
26 chemically analyzed and quantified. Furthermore, as suggested by Ashauer *et al.*
27 (2012), the biotransformation pathways could be misunderstood: indeed, the model is
28 not currently designed to consider metabolites derived from the products of parent-
29 compounds biotransformation (phase II metabolites). This biotransformation pathway
30 can be reasonably expected to decrease uncertainties as the process would be better
31 described than when we hypothesized that all metabolites originated from the parent
32 compound (larger uncertainties). It is therefore crucial to gain a better understanding
33 of the biotransformation cascade mechanisms. Furthermore, analytical results may be
34 more uncertain for metabolites than for parent compounds, due to the lack of
35 standards; in which case the model fit would be accordingly less accurate. Another
36 limitation with this kind of bioaccumulation tests is that they require a lot of
37 measurements, and are time consuming and expensive.

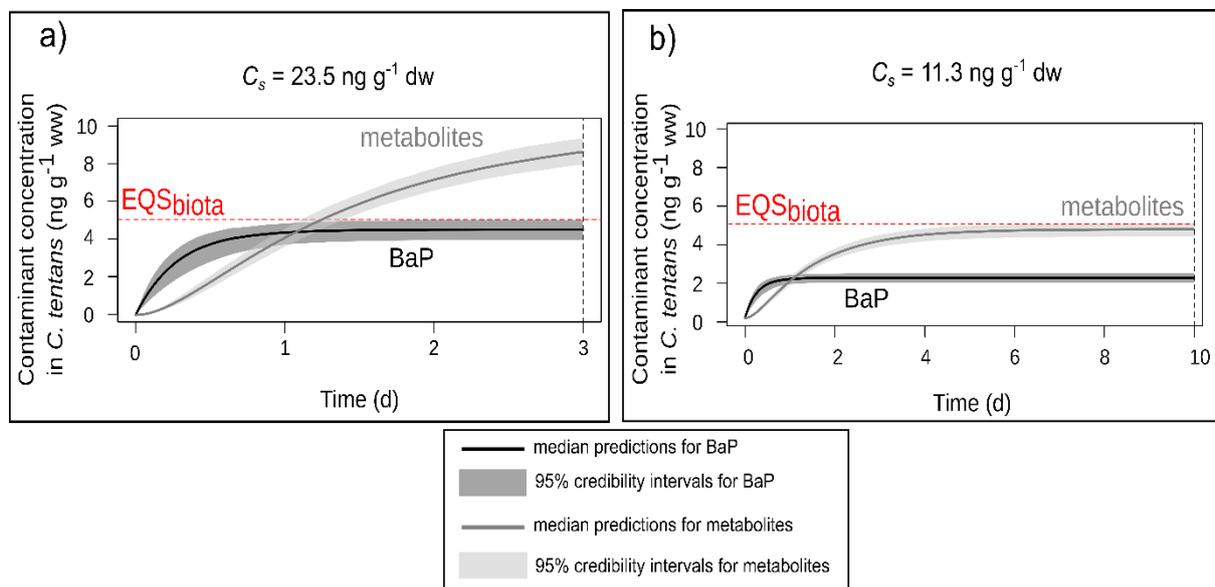
38 **4.2. Applications**

39
40 TK models are used in ERA as the first step to evaluating the toxicity of a
41 contaminant. They are developed to provide a theoretical framework for understanding
42 exposure-uptake relationships and accumulation in organisms, testing hypotheses,
43 and making predictions (e.g. predictions of the chemical concentration in organisms
44 according to environmental concentration or *vice versa*). ERA of organic contaminants,
45 and the ensuing regulatory guidelines for chemicals management and control have

1 mostly been restrained to the bioaccumulation potential of the parent compounds (Kuo
2 and Chen, 2016). However, there is legitimate concern over the failure to regulate for
3 metabolites, which might have more effects than the parent compounds on organisms.

4 Some benthic organisms are able to metabolize and excrete benzo(a)pyrene
5 (BaP) accumulated in their body (Schuler *et al.*, 2003). However, some intermediates
6 of BaP metabolism are highly toxic and highly mutagenic and/or carcinogenic (U.S.
7 EPA., 2017). In France, the EQS_{biota} defined for invertebrates exposed to PAHs is
8 $5.00 \text{ ng g}^{-1} \text{ ww}$ (Directive 2008/105/EC). This value refers to BaP, which is deemed
9 representing all PAHs.

10 Here we illustrate how to use TK models to retro-predict chemical
11 concentrations in the sediment leading to the EQS_{biota} . For this purpose, we first used
12 experimental data from a published TK study on *Chironomus tentans* exposed to BaP
13 spiked sediment (Schuler *et al.* 2003) to estimate the distributions of the model
14 parameters and thus go on to predict the concentration in sediment that would lead to
15 a concentration in biota below the corresponding EQS_{biota} for both BaP and its
16 metabolites. We also spread the uncertainties around the median predictions. The
17 results are reported on Figure 3. The capacity of the chironomids to metabolize BaP is
18 such that the metabolites alone achieve almost double the EQS_{biota} concentration
19 defined for the parent compound (Fig. 3-a). For the in-organism concentration of
20 metabolites to meet EQS_{biota} , the in-sediment concentration of BaP has to be two times
21 lower ($11.3 \text{ ng g}^{-1} \text{ dw}$, Fig. 3-b) than the predicted in-organism concentration for BaP
22 only ($23.5 \text{ ng g}^{-1} \text{ dw}$, Fig. 3-a). We also extended the exposure time (from 3 to 10 days)
23 in order to reach the steady state for metabolite concentration in chironomids (Fig. 3-
24 b).
25



26 **Figure 3.** Predictions for *C. tentans* exposed to BaP spiked sediment (a) at $23.5 \text{ ng g}^{-1} \text{ dw}$ for 3 days and (b) at $11.3 \text{ ng g}^{-1} \text{ dw}$ for 10 days. The EQS_{biota} for BaP ($5.00 \text{ ng g}^{-1} \text{ ww}$) is represented by the red dashed horizontal line.

27 These findings raised the issue of how the regulations account for metabolites, when
28 metabolite concentrations in the organism could exceed the EQS_{biota} defined for the
29 parent compound.

5. Conclusions

Here we proposed a unified inference framework to estimate kinetic parameters of a generic TK model, including biotransformation rates. A Bayesian framework was used to simultaneously estimated all parameters. This approach results in a quantification of the uncertainties around model predictions that play a key role in environmental risk assessment. A total of 22 aquatic invertebrate species-chemicals pairs were tested. The data encompassed different ranges of contaminants including pesticides, drugs and PAHs as well as various biological and ecological traits. Our framework also considers one to several metabolites. We successfully estimated the elimination parameters distinguishing excretion from biotransformation by applying the framework to several invertebrate species and chemicals. This approach is transferable to any species-chemicals pair, and it provides an accurate estimate of elimination rates by distinguishing the classical elimination rate from the biotransformation rate(s). The major added value of the Bayesian approach is to estimate simultaneously model parameter from different kinds of data. The predictions and their uncertainties around concentrations by the joint *a posteriori* distribution will also improve ERA, as illustrated in this study.

6. Outlook

We anticipate that Bayesian analysis can be used to simultaneously estimate parameters considering several exposure routes such as sediment and water. The model might be improved by integrating pulse exposures and secondary metabolite kinetics. Under laboratory conditions (e.g. OECD tests), and in most of the datasets available in the literature, the exposure concentration is usually held constant over time. However, the assumption of constant exposure concentration actually adds another layer of uncertainty, as under real environmental conditions the concentration of the parent compound may vary over time.

A better understanding of biotransformation in invertebrates, especially for new chemicals for which few data are available, is a necessary step towards better risk assessment of chemicals, especially their biological effects in invertebrates and at higher trophic levels. k_m could thus be integrated into aquatic food web models or used to complete databases in other modeling frameworks, such as QSAR modeling (Arnot *et al.*, 2008b). The biotransformation rate could also be integrated in toxicokinetic/toxicodynamic frameworks, such as DEBtox or GUTS models (Ockleford *et al.*, 2018). In parallel, biological investigations such as research to develop biomarkers, could provide further valuable foundations for understanding and modeling biotransformation capacity in invertebrates.

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