Comparison of a simple and a complex model for BCF prediction using *in vitro* biotransformation data

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ABSTRACT

A promising approach for bioaccumulation assessment with reduced animal use is the prediction of bioconcentration factors (BCFs) using *in vitro* biotransformation data. However, it has been recognized that the BCFs predicted using current models often are in poor agreement with experimental BCFs. Furthermore, extrahepatic biotransformation (e.g. in gill or GIT) is usually not accounted for.

Here, we compare two BCF prediction models: a simple one-compartment and a more advanced multi-compartment model. Both models are implemented in a two-in-one calculation tool for the prediction of BCFs using *in vitro* data. Furthermore, both models were set up in a way that *in vitro* data for extrahepatic biotransformation can be easily considered, if desired. The models differ in their complexity: the one-compartment model is attractive because its simplicity, while the multi-compartment model is characterized by its refined closeness to reality. A comparison of the results shows that both models yield almost identical results for the presently evaluated cases with plausible physiological data. For regulatory purposes, there is thus no reason not to use the simple one-compartment model. However, if it is desired to represent special *in vivo* characteristics, e.g. first-pass effects or the direct GIT-to-liver blood flow, the multi-compartment model should be used.

KEYWORDS

Bioaccumulation, Modeling, *In vitro – in vivo* extrapolation, Biotransformation

1. INTRODUCTION

Evaluation of the bioaccumulation potential of chemicals is one crucial aspect for environmental risk assessment. Commonly, the bioaccumulation potential of a chemical is quantified via the fish bioconcentration factor (BCF). The BCF corresponds to the steady-state concentration of the chemical in the organism divided by that in the surrounding water phase. Determination of the BCF for a certain chemical can be either performed experimentally via a fish test or theoretically via the application of prediction models (Arnot and Gobas 2003, Arnot and Gobas 2006). A very simple prediction 'model' that was first proposed by Neely et al. (Neely et al. 1974) calculates a chemical's BCF based on its hydrophobicity (expressed by means of the octanol-water partition coefficient K_{OW}), but these predictions fail when organic compounds are actively metabolized in fish. More advanced prediction models thus calculate the BCF from uptake and elimination rate constants. Such prediction models can be helpful tools for bioaccumulation assessment, especially for high throughput assessments under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). The application of prediction models could serve as an intermediate step between the partition-based first tier-assessment and the animal test as second tier in bioaccumulation assessment.

A particular important parameter in the prediction models is elimination of the chemical via biotransformation inside the organism, because rapid biotransformation can reduce the BCF significantly. In contrast to other elimination processes, the rate of biotransformation cannot be estimated readily from the physico-chemical properties of the compound. Instead, the current method of choice is to perform *in vitro* biotransformation studies and to predict the corresponding *in vivo* kinetics from the generated data (*in vitro-in vivo*-extrapolation). However, when prediction results are compared with *in vivo* bioconcentration tests, significant discrepancies are often reported (Nichols et al. 2013, Fay et al. 2014, Laue et al. 2014). The reasons for these discrepancies are still unclear. In this manuscript, we evaluate two modeling-related issues that might contribute to the observed discrepancies.

Different models can be used for bioaccumulation prediction: simpler models that need less input information and are easy-to-use or more complex models that represent the *in vivo* reality

more accurately. Examples for a more accurate depiction of the *in vivo* reality are the representation of the important sites of biotransformation separated from the rest of the fish and under consideration of their physiological features. Classically, the liver is assumed to be the major site of biotransformation (Binder et al. 1984). However, from the literature it is known that certain extrahepatic tissues like gills and the intestinal cells of the gastro-intestinal tract (GIT) can also exhibit biotransformation capacity (Barron et al. 1989, Gomez et al. 2010). Recently, the awareness of the impact of extrahepatic biotransformation has increased and first studies investigating biotransformation in gill or GIT tissues are available (Stadnicka-Michalak et al. 2018). When gills and GIT are to be considered as additionally important sites of biotransformation, new questions arise. The first question is whether a first-pass effect in the gills may affect the resulting BCF. In contrast to the intensively studied first-pass effect in GIT and liver of mammals following oral administration (Pond and Tozer 1984), less is known about the first-pass in fish gills following chemical uptake via ventilation. However, it was shown for non-steady-state condition that a first-pass effect in fish occurs after chemical uptake via the gills when biotransformation in the gills is so fast that the chemical is already eliminated before it reaches the rest of the body (Levine and Oris 1999). The second question is whether the fact that biotransformation in GIT tissue and liver occurs in sequential order and not in parallel have an influence on the BCF.

We intend to address these questions in the present manuscript by providing a direct comparison of two models. The first model is the simplest model possible: a one-compartment model that neglects any complicating factors (e.g. blood flow limitation). The second one is a more complex model that we developed with the purpose of representing the parallel acting biotransformation activities of liver and GIT as well as the potential first-pass effect in the gills. It considers the metabolically active organs (liver, gills, GIT) as compartments separate from the rest of the body and represents blood flow to all metabolically active tissues in the correct order. We evaluate possible differences in the outcome of both models by applying them to identical, generic scenarios.

Both models are implemented in our supporting 'B-compass fish' (**b**ioaccumulation one-/multi-**compa**rtment **s**teady **s**tate model) calculation tool so that the results of both models can be compared directly. Note that both models can use information on biotransformation in liver, gills and GIT but do not have to – i.e. both models can also be used if only information on biotransformation in one or two tissues is available. Furthermore, both models were set up to allow for consideration of chemical uptake via GIT and, thus, allowing BAFs to be predicted. In this work, however, we will focus exclusively on the application for BCF prediction (water exposure only).

2. METHODS/THEORY

2.1 Model structure

At first, we start with defining the processes that need to be accounted for in the models. In the BCF-scenario, contamination of the organism occurs only via ventilation. Accordingly, the only uptake process that needs to be considered is uptake via ventilation. In contrast, elimination occurs via ventilation, biotransformation in liver, gills and GIT and via fecal egestion. Dilution due to growth has been considered as an additional elimination process in models that have been used for comparison with experimental BCF data. This has recently been put into question by Gobas and Lee (Gobas and Lee 2019) because the performed growth correction violates the mass balance and neglects that the respiration rate (and by this chemical uptake) also increases with increasing fish size. Furthermore, BCFs should preferably be determined in non- or slow-growing animals anyway (OECD 2012). We thus do not consider growth in the here presented models.

As mentioned above, we use two models for BCF prediction: a one-compartment model and a steady-state multi-compartment model. In the one-compartment model, the whole organism is assumed to be a single well-mixed compartment with a homogeneous concentration. All kinetic processes apply to this whole-body concentration, hence they are assumed to act in parallel. The whole-body biotransformation rate constants used in this model do not account for blood flow limitation. However, when extrapolating *in vitro* biotransformation data to whole-body biotransformation rate constants with the approach established by Nichols and coworkers blood flow limitation is already accounted for (Nichols et al. 2006). Strictly thinking, this procedure already adds a level of complexity and goes beyond the concept of a pure one-compartment model, because the existence of flow limitation requires the presence of liver and rest of the body as two separate units with disequilibrium between them. For better discrimination, we thus suggest to use the term ‘quasi-one-compartment model’ for one-compartment models using flow limited biotransformation rate constants. Note that this quasi-one-compartment modeling approach currently is the most commonly used one in the context of BCF prediction based on *in vitro* information for biotransformation (Gomez et al. 2010,

Nichols et al. 2013, Fay et al. 2014, Laue et al. 2014, Nichols et al. 2018). Expansion of the quasi-one-compartment model so that extrahepatic biotransformation is considered is possible, but the *in vivo* complexity with first-pass effects in the gills and the sequential biotransformation activities of GIT and liver cannot be represented easily in this modeling approach. In terms of complexity but also with respect to the expected quantitative outcome, the quasi-one-compartment model will always be an intermediate between the two 'extreme' modeling scenarios described above. We therefore decided to study the extreme endpoints on this scale of differently complex modeling approaches because we considered this to be the most informative evaluation with respect to the possibly different outcomes.

Figure 1 provides a conceptual overview of the different types of models. We also included the scheme of the mentioned quasi-one-compartment model, although this model variant is not considered any further. Note that the only difference between the one-compartment and the quasi-one-compartment model is the use of whole-body biotransformation rate constants that already consider blood flow limitation in the quasi-one-compartment model.

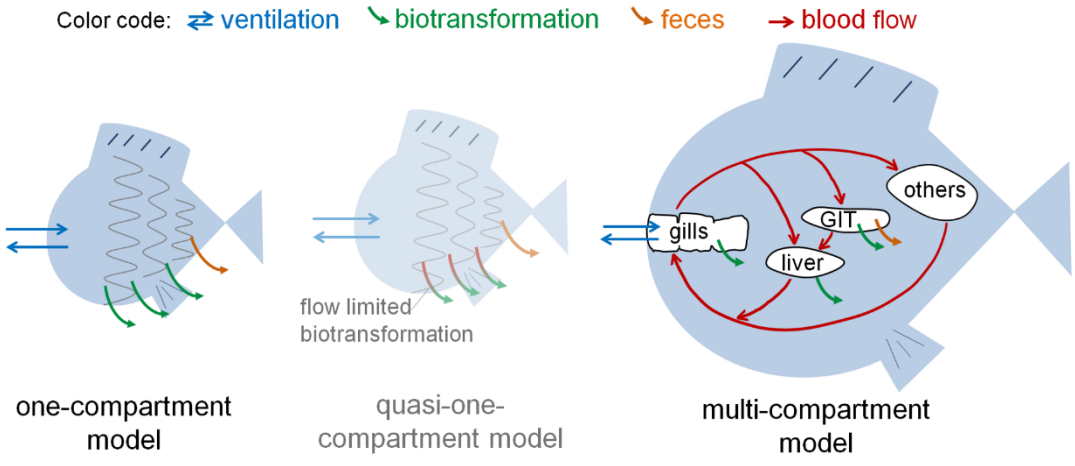

Figure 1: Conceptual overview of the different kinds of models.

The second model that we evaluate in this study explicitly considers four different compartments. These are gills, liver, GIT and rest of the body (called 'others' in Figure 1). Exchange between these compartments occurs via blood flow. All elimination processes, i.e. biotransformation in gills, liver, GIT, fecal egestion from GIT and elimination via ventilation from the gills, apply to the corresponding tissue concentrations instead of the whole-body

concentration. For calculation of the BCF, the steady-state concentrations in the four compartments are calculated separately and, using the corresponding volumes, combined to the whole-body steady-state concentration. We thus call this model a steady-state multi-compartment model.

2.2 Mathematics

Both models rely on mass-balance approaches and are implemented in the supporting 'B-compass fish' excel tool.

2.2.1 One-compartment model

For the one-compartment model, the mass balance for the organism expressed in words (for the mathematical equations see SI section 1a) is as follows

$$\begin{aligned} &chemical\ in\ organism\ over\ time \\ &= uptake\ into\ body\ via\ ventilation \\ &- elimination\ from\ body\ via\ ventilation \\ &- elimination\ from\ body\ via\ gill\ biotansformation \\ &- elimination\ from\ body\ via\ hepatic\ biotransformation \\ &- elimination\ from\ body\ via\ GIT\ biotransformation \\ &- elimination\ from\ body\ via\ fecal\ egestion \end{aligned}$$

For steady-state conditions, this approach yields the following equation for BCF calculation (for details see SI section 1a):

$$BCF_{one-comp.} = \frac{k_1}{(k_V + k_{B,GILLS} + k_{B,LIVER} + k_{B,GIT} + k_E)} \quad (1)$$

Here, k_1 is the ventilation uptake rate constant from the surrounding water ($L_W/kg_{fish}/d$) , k_V is the whole-body elimination rate constant via ventilation (also called branchial elimination rate constant) in 1/d, $k_{B,GILLS}$ is the whole-body gill biotransformation rate constant (1/d), $k_{B,LIVER}$ is the whole-body hepatic biotransformation rate constant (1/d), $k_{B,GIT}$ is the whole-body GIT

biotransformation rate constant (1/d) and k_E is the whole-body fecal egestion rate constant (1/d).

Note that a BCF calculated using eq. **(1)** is based on freely dissolved chemical concentration in the surrounding water. However, it is also possible to calculate BCFs based on total chemical concentration. For details, see SI section 1c. B-compass fish provides both BCFs as output.

2.2.2 Steady-state multi-compartment model

For the multi-compartment model, the chemical in the whole organism is calculated as the sum of the chemical in the different compartments:

$$chemical\ in\ organism\ over\ time = chemical\ in\ gills\ over\ time + chemical\ in\ liver\ over\ time + chemical\ in\ GIT\ over\ time + chemical\ in\ rest\ body\ over\ time$$

For the different compartments, separate mass balance approaches are formulated. Details and the resulting equation for BCF calculation can be found in section 1b and 1c of the SI.

2.3 Input data

2.3.1 General

Both presented BCF prediction models require information on the partition properties of the chemical and on the physiological parameters of the animal. The required partition coefficients can be either calculated using poly-parameter free energy relationships (ppLFERs) (Endo et al. 2013) or they can be deduced from log K_{OW}-correlations. Our method of choice for prediction of partition coefficients is the more accurate ppLFER-approach (Endo et al. 2013), however, the prediction using log K_{OW} correlations is more frequently used. We thus focus on the latter one in the main text, details for the ppLFER approach are provided in the SI (section 2a). The implementation into the B-compass fish tool is provided for both approaches.

An overview how the required partition information is calculated from K_{OW} can be found in Table 1. For these calculations, it is assumed that the octanol-water partition coefficient multiplied with an adjustment factor of 0.05 is able to describe partitioning into proteins and

that the octanol-water partitioning without any adjustment factor is a suitable surrogate to describe partitioning into lipids.

Table 1: Calculation of the required partition information using K_{OW} correlations

Parameter	Symbol (unit)	Equation or value	reference
unbound fraction in *in vitro* assay	$f_{u,assay}$ (unitless)	$\frac{w_{assay}}{w_{assay} + protein_{assay} * 0.05K_{OW} + lipid_{assay} * K_{OW}}$	(Lee et al. 2017)
unbound fraction in blood	$f_{u,blood}$ (unitless)	$\frac{w_{blood}}{w_{blood} + protein_{blood} * 0.05K_{OW} + lipid_{blood} * K_{OW}}$	(Lee et al. 2017)
blood-water partition coefficient	$K_{blood/water}$ (mL_{water}/mL_{blood})	$w_{blood} + protein_{blood} * 0.05K_{OW} + lipid_{blood} * K_{OW}$	adapted from (Lee et al. 2017)
organism-water partition coefficient	$K_{organism/water}$ ($mL_{water}/mL_{organism}$)	$lipid_{organism} * K_{OW}$	

The water, protein and lipid contents of the assay (w_{assay}, $protein_{assay}$ and $lipid_{assay}$) are calculated from the used S9 or cell concentration and the water, protein and lipid contents of blood (w_{blood}, $protein_{blood}$ and $lipid_{blood}$) can be found in literature (for details see SI section 2b). The lipid content of the organism ($lipid_{organism}$) is assumed to be 5 %.

The models also need physiological parameters comprising scaling factors. Due to the lack of published scaling factors for GIT S9 and gill S9, we use the S9 content of liver as surrogate. Furthermore, the models need the bodyweight of the modeled fish and the modeled temperature as input data. The temperature is required in the algorithm for prediction of the fecal egestion rate constant k_E (see Table 2) and for prediction of the cardiac output that is needed for representation of blood flow limitation in the multi-compartment model. Temperature-dependencies of other input data (e.g. the partition coefficients) are not

accounted for because algorithms for temperature-dependent predictions of these data are not available yet. For all calculations presented here, we use a fish bodyweight (m_{body}) of 10 g with 5 % lipid and a temperature (T) of 15 °C. A list of all required physiological parameters can be found in SI section 2b in table S5.

Besides that, information on the uptake and elimination processes is needed. In the following, we present how the kinetic information is derived that is needed for each model.

2.3.2 One-compartment model

The input parameters required for the one-compartment model are the whole-body rate constants for uptake and elimination. The uptake rate constant k_1, the whole-body ventilation elimination rate constant k_V and the whole-body fecal egestion rate constant k_E can be estimated from published empirical equations (see Table 2). Among these, the uptake rate constant k_1 is often regarded as a source of noteworthy uncertainty; we thus offer two variants for estimation of k_1 in B-compass fish: one from Arnot and Gobas (Arnot and Gobas 2003) and the other from Sijm et al (Sijm et al. 1995). By default, the k_1 algorithm from Sijm et al. (Sijm et al. 1995) which is also referred to in the current OECD 305 document is preselected in B-compass fish.

Table 2: Used equations for estimation of k_1, k_V and k_E

Parameter	Symbol (unit)	Equation or value	reference
uptake rate constant	k_1 ($L_W/kg_{fish}/d$)	$520 * m_{body}^{-0.32}$ or $\frac{1}{\left(0.01+\frac{1}{K_{OW}}\right)*\left(\frac{m_{body}}{1000}\right)^{0.4}}$	(Sijm et al. 1995) or (Arnot and Gobas 2003)
whole-body ventilation rate constant	k_V (1/d)	$\frac{k_1}{K_{organism/water}}$	(Arnot and Gobas 2003)
whole-body fecal egestion rate constant	k_E (1/d)	$\frac{0.125 * (0.02 * (m_{body}/1000) - 0.15 * e^{0.06T})}{5.1 * 10^{-8} * K_{OW} + 2}$	(Arnot and Gobas 2003)

The whole-body liver, gill and GIT biotransformation rate constants are derived via extrapolation of corresponding *in vitro* information. For the example of *in vitro* assays with S9 material (either isolated from liver, GIT or gills), we extrapolate in two steps (Krause and Goss 2018). The first one is the extrapolation from assay to blood clearance without flow limitation $CL_{blood\ w/o\ flow\ lim}$ ($mL_{blood}/h/g_{fish}$):

$$CL_{blood\ w/o\ flow\ lim} = \frac{f_{blood}^{unbound}}{f_{assay}^{unbound}} * k_{in\ vitro} * \frac{C_{S9\ in\ organism}}{C_{S9\ in\ assay}} * \frac{w_{assay}}{w_{blood}} \quad (2)$$

The second one is the extrapolation to the needed whole-body biotransformation rate constant (1/h):

$$k_{B,LIVER\ or\ GILLS\ or\ GIT} = CL_{blood\ w/o\ flow\ lim} * \frac{K_{blood/water}}{K_{organism/water}} \quad (3)$$

Here, $CL_{blood\ w/o\ flow\ lim}$ ($mL_{blood}/h/g_{fish}$) is the bodyweight-normalized blood clearance due to biotransformation in liver, gills or GIT without flow limitation, $\frac{f_{blood}^{unbound}}{f_{assay}^{unbound}}$ is the ratio of unbound fractions in blood and assay (unitless), $k_{in\ vitro}$ is the rate constant determined in the liver, GIT or gill S9 *in vitro* assay (1/h), $C_{S9\ in\ assay}$ is the S9 concentration used in the *in vitro* assay (mg_{S9}/mL_{assay}), $C_{S9\ in\ organism}$ is the S9 concentration in body in mg_{S9}/g_{fish} (given by the S9 content of the respective tissue and the respective tissue weight as fraction of bodyweight), $\frac{w_{assay}}{w_{blood}}$ is the ratio of water contents in assay and blood $\left(\frac{mL_{water}/mL_{assay}}{mL_{water}/mL_{blood}}\right)$, $k_{B,LIVER\ or\ GILLS\ or\ GIT}$ is the liver, gills or GIT whole-body biotransformation rate constant (1/h) and $K_{blood/water}$ and $K_{organism/water}$ are the blood-water and organism-water partition coefficients of the chemical (mL_{water}/mL_{blood} and $mL_{water}/g_{organism}$). Note that the ratio $K_{blood/water}$ and $K_{organism/water}$ corresponds to the inverse of the 'volume of distribution', how the organism-blood partition coefficient is often called. A density of the organism of 1 g/mL is assumed. Note that instead of this two-step extrapolation procedure one could also extrapolate directly from assay to whole organism (see SI section 2c and (Krause and Goss 2018)). We decided to provide the two-step extrapolation here because

this is the commonly used procedure. A detailed discussion of the manifold extrapolation paths can be found in a recent paper (Krause and Goss 2018).

For *in vitro* assays with cells instead of S9 material, the equation for extrapolation is analogue but uses cell concentrations instead of S9 concentrations (see SI section 2d).

2.3.3 Steady-state multi-compartment model

In multi-compartment model, the required uptake and elimination rate constants refer to the tissue concentrations instead of whole-body concentration. For doing so, the rate constants are principally calculated as presented above but need to be modified in their normalizations (see SI section 2e for details). Alternatively, the required biotransformation rate constants could also be calculated directly using a correspondingly adjusted extrapolation procedure (see SI section 2e).

Furthermore, the multi-compartment model represents compound exchange between the different organs via transport with blood flow. Accordingly, organ blood flow rates are required as additional input parameters. These organ blood flow rates are calculated from the cardiac output that is given by the allometric formula $(0.23 * T - 0.78) * (m_{body}/500)^{-0.1} * 24$ from Erickson and McKim (Erickson and McKim 1990). In the B-compass fish tool, we provided rainbow trout specific default values for all parameters that are additionally required for application of the multi-compartment model (see SI section 2f for further details). By this, the user can apply the multi-compartment model without any additional effort.

3. RESULTS & DISCUSSION

3.1 Differences between the models and outlook on the expected consequences

As mentioned above, the one-compartment model neglects any blood flow limitation effects. Accordingly, the one-compartment model is expected to yield lower BCFs than the multi-compartment model in case of fast intrinsic biotransformation. This potential error would not lead to a worst case assessment and may therefore not appear acceptable from the regulative

perspective. However, a strong blood flow limitation is only likely to happen for chemicals with fast biotransformation which are less likely to be bioaccumulative in the first place.

Above we also stressed that the multi-compartment approach allows the first-pass effect in the gills to be taken into account and represents the direct GIT-to-liver blood flow. Due to the first-pass effect lower chemical concentrations in the rest of the body and, by this, lower BCFs might result. The consequences of the direct GIT-to-liver blood flow can also be anticipated: In case GIT biotransformation leads to a notable reduction of the chemical concentration, the subsequent hepatic biotransformation applies to a lower blood concentration. Accordingly, the amount of chemical that is eliminated via hepatic biotransformation in the multi-compartment model can be lower than in the one-compartment model. The combined effect of these processes cannot be generally predicted a priori; we thus evaluated the combined effects via a systematic comparison of both models for varying input parameters covering a realistic range.

3.2 Comparison of BCFs calculated for varying input parameters

For a comparison of the models, we considered scenarios with varying input parameters. The parameters that were varied are the following:

- hepatic biotransformation rate constant
- gill biotransformation rate constant
- GIT biotransformation rate constant
- hydrophobicity

To evaluate the impact of the different biotransformation kinetics, we calculated the BCFs with both models for slow, intermediate and fast biotransformation rate constants (0.1 h^{-1}, 1 h^{-1} and 10 h^{-1}) (Halder et al. 2018). Furthermore, we varied the octanol-water partition coefficient of the chemical as a measure of hydrophobicity (log K_{OW} of 4.5, 5.5 and 6.5).

Note that, to better illustrate the influence of a certain parameter on the result, we varied only one parameter at a time and used fixed ‘standard’ values for the other parameters. These ‘standard’ values were log K_{OW} = 5.5, and *in vitro* cell assay rate constants of $k_{hep\text{-}assay}$ = 0.1 h^{-}

[1], $k_{gill\text{-}assay}$ = 0.1 h^{-1}, $k_{GIT\text{-}assay}$ = 0.1 h^{-1}. For fish bodyweight and holding temperature fixed values were used for all calculations, these were 10 g (with 5 % lipid) and 15 °C. The uptake via ventilation is predicted using the k_1 algorithm from Arnot and Gobas (Arnot and Gobas 2003). The results are compared as BCFs based on freely dissolved chemical concentrations (Table 3) and are shortly discussed in the following.

Table 3: Comparison of BCFs calculated with the one-compartment and the multi-compartment model for varying input parameters

varied input parameter		resulting BCFs [L/kg] one-compartment model	resulting BCFs [L/kg] multi-compartment model
hepatic biotransformation rate constant [1/h]	0.1	2570	2578
	1	1032	1066
	10	148	208
GIT biotransformation rate constant [1/h]	0.1	2570	2578
	1	877	919
	10	116	183
gill biotransformation rate constant [1/h]	0.1	2570	2578
	1	525	525
	10	59	59
log K_{OW} of the chemical [L/L]	4.5	1013	1014
	5.5	2570	2578
	6.5	3042	3053

The first result for varying hepatic biotransformation is really intuitive: The higher the hepatic biotransformation rate constant, the lower the resulting BCFs. Independently of the used hepatic biotransformation constant, the one-compartment model always yields lower BCFs than the multi-compartment model (Table 3). This result matches the expectations: Because the one-compartment model neglects any blood flow limitation, the elimination via biotransformation is slightly higher than in the other model where flow limitation is accounted for. The differences between the calculated BCF values are rather small for slow and intermediate hepatic biotransformation rate constants, but for the fast hepatic biotransformation rate constant the difference increases (30 % lower than the BCF calculated

329 with the multi-compartment model). The reason for this observation is that blood flow limitation
330 becomes most important in case of fast biotransformation.
331 The same can be observed for GIT biotransformation: For the slow and intermediate GIT
332 biotransformation rate constant, the differences between the models again are rather small
333 and the one-compartment model yields lower BCFs. For the fast GIT biotransformation rate
334 constant, the differences increase (roughly 40 %). The explanation again is the neglect of blood
335 flow limitation in the one-compartment model.
336 For gill biotransformation, the differences between the models are now small for all evaluated
337 biotransformation rate constants (i.e. the slow, intermediate and fast biotransformation rate
338 constant). Different than expected, the first-pass effect does not have a strong influence on the
339 resulting BCFs. This can be explained as follows: As mentioned above gill biotransformation
340 needs to be faster than gill blood flow for a first-pass effect to occur. However, the physiological
341 blood flow is so fast that this situation rarely occurs. When this situation occurs,
342 biotransformation is so fast that the resulting BCFs are already very low and no differences
343 between the models can be observed.
344 When one compares the impact of biotransformation in liver, GIT and gill among each other,
345 one can notice that different BCFs result depending on whether the *in vitro* rate constant for
346 liver, GIT or gill increased, e.g. a gill *in vitro* biotransformation rate constant of 10 h^{-1} yields a
347 lower BCF then a hepatic biotransformation rate constant of 10 h^{-1}. The reason for this is that
348 the different scaling factors for liver, GIT and gill (cell content per g tissue and organ weight
349 per bodyweight in this example) lead to different *in vivo* rate constants even when *in vitro* rate
350 constants have identical values. The here presented generic analysis does not allow any
351 conclusions on whether biotransformation in one tissue is more important than that in another
352 tissue, because hypothetical combinations of *in vitro* rate constants were used. Such kind of
353 conclusions are only possible when experimental values for the *in vitro* rate constants in the
354 three tissues are available for the compound of interest (see benzo(a)pyrene example below).
355 For varying octanol-water partition coefficients, the first and obvious result is that with
356 increasing log K_{OW} the calculated BCFs also increase. The relative differences between the

models for a given log K_{OW} are small (<1 % difference) with the one-compartment model yielding slightly lower BCFs than the multi-compartment model because of blood flow limitation.

3.3 Application of both models for experimental data from the literature

To evaluate the performance of both models with experimental input data, we used recently published results on biotransformation of benzo(a)pyrene in liver, gills and GIT (Stadnicka-Michalak et al. 2018) to predict the corresponding BCFs based on total concentration. A detailed summary of the used input data can be found in SI section 3. The uptake kinetics are calculated using the k_1-algorithm from Sijm.

The results of both our models using the *in vitro* data from the study by Stadnicka et al. (Stadnicka-Michalak et al. 2018) are presented in Table 5.

Table 4: BCFs [L/kg] predicted from *in vitro* biotransformation data for biotransformation in liver, GIT and gills compared in comparison with the BCFs predicted with PBTK model in (Stadnicka-Michalak et al. 2018) for a 10 g fish and an experimental *in vivo* BCF

test chemical	log K_{OW}	$CL_{in\ vitro,int}$ (mL/h/10^6 cells)	predicted BCFs			experimental in vivo BCF from (Stadnicka-Michalak et al. 2018)
			one-comp.	multi-comp.	PBTK model from (Stadnicka-Michalak et al. 2018)	
benzo(a)pyrene	6.13	liver: 0.43 GIT: 0.27 gill: 0.07	620	624	1126	920

For benzo(a)pyrene, the difference between the one-compartment model and the multi-compartment model is small. Again, the one-compartment model yields a slightly lower BCF than the multi-compartment model because of light blood flow limitation. The BCFs predicted with the here presented models are lower than the BCF predicted by Stadnicka et al. (Stadnicka-Michalak et al. 2018). These differences can be explained as follows: i) in the literature, the extrapolation of the *in vitro* data was not yet performed with the revised extrapolation procedure that considers the water contents *in vitro* and *in vivo* and yields higher *in vivo* rate constants (Krause and Goss 2018), ii) the partition information, i.e. unbound

fractions in assay and blood and the required partition coefficients, was calculated with different algorithms and iii) slightly different physiological data was used.

As an additional output, the B-compass fish calculation tool illustrates the relevance of the different elimination processes in both models in supporting graphs. The graphs rely on mass flow information and show the steady-state elimination expressed as percent of the chemical mass taken up via ventilation. Figure 2 shows the graph for benzo(a)pyrene calculated the *in vitro* biotransformation data from Stadnicka et al. (Stadnicka-Michalak et al. 2018).

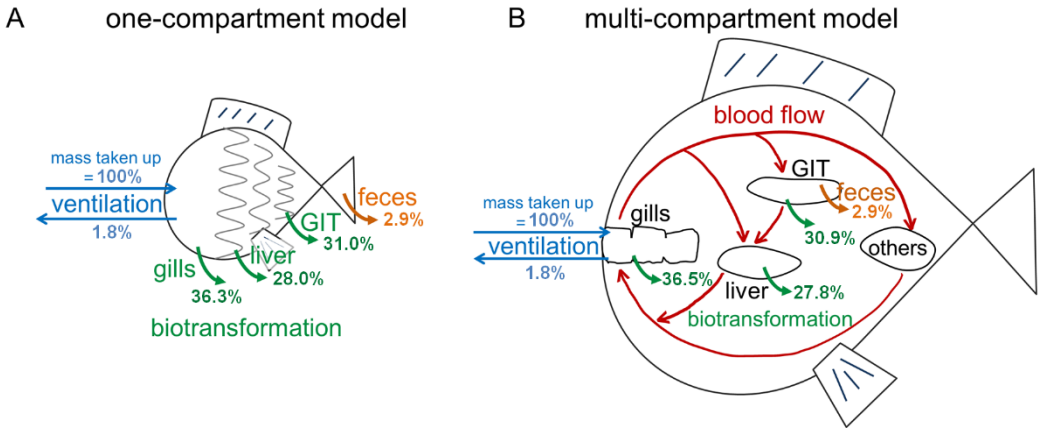

Figure 2: The pathways of benzo(a)pyrene elimination in the one-compartment model (A) and in the multi-compartment model (B) expressed as percent of the chemical taken up via ventilation per day. Recently published *in vitro* biotransformation data for GIT, liver and gills were used.

In part A of Figure 2 the results of the one-compartment model are shown. It can be seen that in this case liver and GIT contribute roughly equally to overall elimination, while gill biotransformation contributes slightly more. In the multi-compartment model, the same pattern shows (Figure 2B).

3.4 ‚Reverse' Modeling

The above presented comparisons show that the results of both models are surprisingly similar for most scenarios. From a regulatory perspective, there is thus not much justification for using the multi-compartment model. The one-compartment model should be sufficient. On this basis, one can even do some kind of 'reverse modeling', i.e. one can calculate how fast the whole-body elimination kinetics has to be in order to keep the BCF below the threshold. Assuming that the steady-state concentration in the fish is mainly governed by ventilation (k_1 according

to the Sijm algorithm (Sijm et al. 1995)) and biotransformation in only one tissue, for example the liver, one can deduce the required intrinsic biotransformation kinetics from this whole-body elimination kinetics. This procedure allows to derive the following relationship between the log K_{OW} of a chemical and the intrinsic biotransformation rate constant (Figure 3):

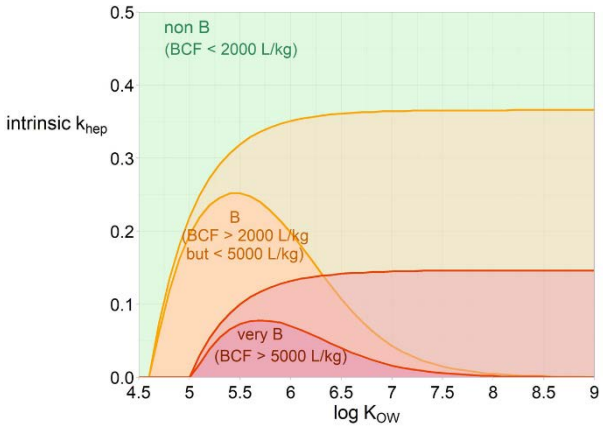

Figure 3: Plot of the different biotransformation rate constants that are required to keep the BCF within certain limits depending on the log K_{OW} of the chemical. The required biotransformation rate constants differ depending on whether the BCF is based on freely dissolved chemical concentration (shaded areas) or total chemical concentration (i.e. consistent with OECD 305 guideline, filled areas).

This graph shows how the BCF of a 10 g-fish with 5% body lipid is related to the intrinsic biotransformation rate constant. Note that we included both BCF 'scenarios' in this graph, i.e. the BCF based on freely dissolved chemical concentration (shaded areas) and the BCF based on total chemical concentration (i.e. consistent with OECD 305 guideline, filled areas). The intrinsic biotransformation rate constant reflects the capacity of hepatocytes to transform the chemical that they contain. This intrinsic biotransformation rate constant can be calculated via extrapolation from *in vitro* rate constants that are determined in hepatocyte or liver S9 incubations. For a rough estimation, the information required for extrapolation can be summed up to 'extrapolation factors': intrinsic k_{hep} = 1.2 * $k_{hepassay}$ or = 0.8 * $k_{liverS9assay}$, respectively. (Assuming standard assay conditions, i.e. hepatocyte concentration = 2 * 10^6 cells/mL, viability = 0.85, S9 concentration = 1 mg_{S9}/mL, total assay volume = 1 mL.) Detailed information on how these 'extrapolation factors' were derived can be found in section 4 of the SI.

By this, Figure 3 can give a first indication whether a chemical bioaccumulates or not only from known log K_{OW} and *in vitro* biotransformation kinetics, i.e. without the application of any model.

Taking into account the number of assumptions and simplifications used for derivation of this graph, we recommend using this graph only for a first orientation but not for any regulatory decisions. Furthermore, Figure 3 impressively illustrates the differences related to the question whether a BCF is determined based on total chemical concentration or freely dissolved chemical concentration in the fish tank water.

Note that as mentioned above, we use the Sijm algorithm (Sijm et al. 1995) for k_1 for this graph. Alternatively, one can also use other algorithms for calculation of k_1 which then leads to different results (see SI section 5 for an alternative version of generated using the k_1 algorithm of Arnot and Gobas (Arnot and Gobas 2003)).

4. CONCLUSION

The presented one-compartment and multi-compartment model for BCF prediction are implemented in the provided 'B-compass fish' excel tool (supporting B-compass fish.xlsm file). By this, direct comparison between the models with identical input data is possible with little effort for the user. The purpose of these comparisons was to evaluate whether the gill first-pass effect or the direct GIT-to-liver blood flow that are only represented in the multi-compartment model have an influence on the predicted BCF.

In the here presented evaluation both models show similar results for most cases. Accordingly, the first-pass effect in the gills and the direct GIT-to-liver blood flow appear to be of minor relevance for the BCF scenario. However, the neglect of blood flow limitation in the simple one-compartment model leads to underestimation of the BCF in case of fast biotransformation of the chemical. Due to the fast biotransformation, the BCFs for these cases are usually low and the underestimation of the BCF with the one-compartment model should thus not be problematic in terms of regulatory decisions. Despite the fact, that both models provide similar results especially for cases with little or no biotransformation, the multi-compartment model might be useful because it always provides a more precise result based on the given input data at no additional cost as compared to the one-compartment model.

SUPPORTING INFORMATION

The B-compass fish calculation tool can be downloaded for free from www.ufz.de/b-compass-fish either as Kow-based version of as ppLFER-based version. Furthermore, details on the two BCF models, a complete overview of the required input data, a summary of the used experimental input data from the literature, details on the derivation of the 'extrapolation factors' used for the reverse modeling and the results of the reverse modeling using a different k_1 algorithm are provided in a supporting pdf file.

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