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PARAMETER UNCERTAINTY IN MODELING BIOACCUMULATION FACTORS OF FISH

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Abstract—We quantified the uncertainty due to biota-related parameters in estimated bioaccumulation factors (BAFs) of persistent organic pollutants for fish through Monte Carlo simulations. For this purpose, the bioaccumulation model OMEGA (Optimal Modeling for EcotoxicoloGical Applications) was parameterized based on data from the existing literature, analysis of allometric data, and maximum likelihood estimation. Lipid contents, fractions of food assimilated, the allometric rate exponent, normalized food intakes, respiration and growth dilution rates, and partial mass transfer resistances in water and lipid layers were included as uncertain parameters. The uncertainty in partial resistances was particularly important in the estimation of the rate constants for chemical intake from water by fish. Uncertainties in the fractions of food assimilated and partial water layer resistances from and to food were particularly important in the estimation of the rate constants of chemical intake from food. The uncertainty in the model outcomes for the bioaccumulation factors for fish was a factor of 10 (ratio of 95th and fifth percentile estimates), which was mainly caused by the uncertainty in the lipid fraction. For chemicals with a K_{OW} of 10^3 to 10^6, the uncertainty in the lipid contents of fish accounted for more than 50% of the uncertainty in the estimated bioaccumulation factor. For chemicals with a high K_{OW} (10^7 and higher), the fractions of food assimilated and partial resistances also contributed to uncertainty in the estimated bioaccumulation factor (up to 60%). A case study showed that uncertainty in estimated BAF for nonpersistent substances can be dominated by uncertainty in the rate constants for metabolic transformation. Environ. Toxicol. Chem. 2011;30:403–412. © 2010 SETAC

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INTRODUCTION

Bioaccumulation is often used as a hazard indicator in environmental risk assessment. Estimations by one-compartment mechanistic bioaccumulation models have been shown to be in reasonable agreement with measured bioaccumulation [1–3]. These models predict accumulation levels on the basis of first-order uptake and elimination kinetics, chemical partitioning, and ideally metabolic transformation. Rate constants describing uptake and elimination can be calculated based on measurements. Basing rate constants on measurements has the advantage of reducing the sources of model uncertainty and enabling more precise predictions to be made. However, an increasing number of risk assessment situations call for bioaccumulation estimates that are applicable to a wide range of species and substances. Instead of using measurements, rate constants can also be estimated based on the flows of water, air, and food through the organisms. Models of these flows of water, air, and food are parameterized by species-specific calibration [4–5] or by allometric relations [6–7]. Whereas reliance on allometric regressions has the advantage of making the model applicable to a wide range of species and substances, it also increases the uncertainty in the model by using parameters, such as diffusion resistances, that cannot always be measured but must be estimated, such as by fitting procedures. For example, the fitting of parameters can be done by weighted least squares so that the estimates match measurements [8]. More sophisticated approaches, such as Markov chain Monte Carlo simulations, Bayesian estimates, or linear inverse modeling, have also been applied to parameterize bioaccumulation models [9–11]. These approaches combine parameterization with uncertainty assessment. Generally, these methods rely on case-specific measurement data, which often increases the amount of input data needed [10].

Uncertainties in species characteristics, substance characteristics, and uptake rate constants have also been propagated to uncertainty in bioaccumulation estimates, mostly by Monte Carlo simulation, where the probability distributions were often derived from location-specific values and literature ranges (e.g., [9,12]) or by default variation around the typical value [13–15]. Identifying the largest sources of uncertainty in bioaccumulation modelling further improves the model development by allowing researchers to focus on the most rewarding improvement options. Identification of the largest sources of uncertainty provides scientists with indications to direct further research. In addition, policy makers must decide how to deal with uncertainty; however, most current legislation does not require uncertainty to be explicitly addressed. Quantifying uncertainty provides policy makers with an indication of the order of magnitude of the uncertainty in model estimates. If the uncertainty in an estimated bioaccumulation factor (BAF) is known, then policy makers can take this into account, for example, by taking a more or less conservative value. When they know the parameter from which the largest uncertainties arise, risk assessors can decide whether additional measurements are required to reduce the uncertainty.

All Supplemental Data may be found in the online version of this article.

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Thus far, no study has investigated the uncertainty in rate constants that were derived from calibrating resistances and allometric relations with field data, taking into account the mutual dependence between parameters and their uncertainties. The aim of this research is to derive the biota-related parameter uncertainty in estimated BAFs for fish by assessing the uncertainties in estimated rate constants and species properties and to determine which factors are dominant. Modeled BAFs and the variances therein were compared to measured BAFs for a range of persistent organic substances. The model OMEGA (Optimal Modelling for EcotoxicoloGical Applications) was used for that purpose.

MATERIALS AND METHODS

Model description

The accumulation of a neutral organic compound X in an organism is defined as the ratio between the concentration in the organism ($C_{X,i}$) and the truly dissolved concentration in water ($C_{X,0}$). The OMEGA model estimates accumulation in food chains [2,16–17]. The model is based on the same principles used in similar models [1,3]. In contrast to other models, in this one the basic physiological flows, such as food intake, water absorption, and production, are related to the size and trophic level of the species. In OMEGA, parameters are calibrated on data sets covering a large number of species.

To assess the uncertainty in BAF estimations of persistent organic compounds for fish, this research focused on the third trophic levels in an aquatic food chain: primary carnivores. To calculate food intakes, the lower trophic levels, i.e., algae and invertebrates, are also included. OMEGA calculates steady-state chemical BAFs in biota as the sum of chemical influx by water ($k_{X,\mathrm{in,w}}$) and uptake from food ($k_{X,\mathrm{in,f}}$) divided by the sum of all elimination rates (Eqn. 1). Units and symbols are summarized in Table 1, and a more detailed description of the model is provided in the Supplemental Data.

$$\mathrm{BAF} = \frac{C_{X,i}}{C_{X,0}} = \frac{k_{X,\mathrm{in,w}} \cdot C_{X,0} + k_{X,\mathrm{in,f}} \cdot C_{X,\mathrm{food}}}{(k_{X,\mathrm{out,w}} + k_{X,\mathrm{out,f}} + k_{X,\mathrm{out,p}})C_{X,0}} \qquad (1)$$

where $C_{X,\mathrm{food}}$ is the concentration of a substance in the food of the organism, $k_{X,\mathrm{out,w}}$ is the rate of chemical elimination by excretion in water, $k_{X,\mathrm{out,f}}$ is the rate of elimination by egestion of substances with feces, and $k_{\mathrm{out},p}$ is the rate of elimination by dilution of the substance concentration in the organism by biomass production. Rate constants for chemical influx rates $k_{X,\mathrm{in},j}$ are calculated according to Equation 2

$$k_{X,\mathrm{in},j} = \frac{(m_i/m^*)^{-b}}{\rho_{\mathrm{H_2O}} + \rho_{\mathrm{CH_2}}/K_{\mathrm{OW}_x} + 1/a_j} \cdot \frac{1}{r} \qquad (2)$$

where m_i/m^* is the mass of the organism relative to a reference mass of 1 kg, and the a_j indicates the transport coefficients, either for water, a_w, or for food, a_f. The influx of substances with water or food is modeled from three components. First, advective transport with food intake or water absorption is limited by the supply of new food or water, which is represented by the inverse of the transport coefficients a_w for water and a_f for food. These physiological flows through an organism scale with the relative mass of the organism (m_i) with the allometric rate exponent b [18]. The diffusion of a substance is limited by the mass transfer resistances in water layers and in lipid layers (second and third component). Partial resistance for diffusion through water layers ($\rho_{\mathrm{H_2O}}$) is assumed to be independent of the substance and equal for all octanol–water partition coefficients (K_OW). Two partial water-layer resistances are distinguished: the resistance from and to water ($\rho_{\mathrm{H_2O,w}}$), used to calculate substance intake rates from water ($k_{X,\mathrm{in,w}}$); and the resistance from and to food ($\rho_{\mathrm{H_2O,f}}$), used for calculation of substance intake from food ($k_{X,\mathrm{in,f}}$). The resistance for diffusion through lipid layers $\rho_{\mathrm{CH_2}}$ scales with the K_OW of a substance. This resistance is encountered during the intake of the substance from water and food. The more hydrophobic a substance is, the less resistance it encounters when passing the lipid layer, which is reflected in the K_OW in the denominator (Eqn. 2). In addition, the partial lipid-layer resistance for permeation is higher in plants than in animals [16], probably reflecting the additional barrier of cell walls in plants ($\rho_{\mathrm{CH_2,0}}$).

For food, a correction factor ($1/r$) is added for the part of the substance that is retained in the food, which is calculated

Table 1. Description of the parameters used for bioaccumulation estimation

Symbol	Description	Unit
K_{OW_x}	Octanol/water partition coefficient of substance X	[–]
m_i	Species weight of organism of trophic level i	kg
m^*	Reference mass of 1 kg	kg
$p_{\mathrm{CH_2}}$	Lipid fraction of organism	kg/kg
p_f	Fraction of food assimilated by fish	kg/kg
$p_{\mathrm{f,2}}$	Fraction of food assimilated by food of fish	kg/kg
$\rho_{\mathrm{CH_2,0}}$	Lipid-layer resistance for plants	kg ·d · kg^{-1}
$\rho_{\mathrm{CH_2}}$	Lipid-layer resistance for animals	kg ·d · kg^{-1}
$\rho_{\mathrm{H_2O,w}}$	Water-layer resistance to and from water	kg ·d · kg^{-1}
$\rho_{\mathrm{H_2O,f}}$	Water-layer resistance to and from food	kg ·d · kg^{-1}
b	Rate exponent	[-]
a_w	Water absorption–excretion coefficient	L· kg^{-1} d^{-1}
a_f	Food ingestion coefficient	kg· kg^{-1} d^{-1}
a_p	Biomass (re)production coefficient	kg· kg^{-1} d^{-1}
$k_{X,\mathrm{in,w}}$	Rate constant for absorption of substance X from water	L · kg^{-1} d^{-1}
$k_{X,\mathrm{in,f}}$	Rate constant for assimilation of substance X from food	kg· kg^{-1} d^{-1}
$k_{X,\mathrm{out,w}}$	Rate constant for elimination of substance X via water	d^{-1}
$k_{X,\mathrm{out,f}}$	Rate constant for elimination of substance X via feces	d^{-1}
$k_{\mathrm{out},p}$	Rate constant for dilution by biomass (re)production	d^{-1}
$k_{\mathrm{X,out}}$	Total elimination rate constant of substance X (sum of $k_{X,\mathrm{out,w}}$, $k_{X,\mathrm{out,f}}$, $k_{\mathrm{out},p}$)	d^{-1}
$p_{X,\mathrm{f}}$	Fraction of substance X assimilated from food	kg/kg
$C_{X,i}$	Concentration of substance X in organism of trophic level i	kg/kg
$C_{X,0}$	Dissolved concentration of substance X in water	kg/L
$C_{X,\mathrm{food}}$	Concentration of substance X in food	kg/kg

according to Equation 3

$$\frac{1}{r} = \frac{p_f}{1-p_f} \cdot \frac{1}{p_{CH_2,food} \cdot (K_{OW_x} - 1) + 1} \quad (3)$$

where p_f is the fraction of food assimilated, and $p_{CH_2,food}$ is the lipid fraction of the food. For water, the correction factor is not required; it is replaced by the value of 1 in Equation 2.

The efflux rates of chemicals through water and feces ($k_{X,out,w}$ and $k_{X,out,f}$) are calculated using the same resistances and flows as for the chemical influx rates, but they are multiplied by a factor that reflects the affinity of the substance for staying in the lipid tissue of an organism (Eqn. 4). This ratio can be interpreted as a partition coefficient between tissue and water.

$$k_{X,out,j} = \frac{1}{p_{CH_2} \cdot (K_{OW_x} - 1)} \cdot k_{X,in,j} \quad (4)$$

where p_{CH_2} is the lipid fraction of the organism.

Substance elimination by dilution because of biomass production ($k_{out,p}$) is calculated according to Equation 5

$$k_{out,p} = a_p \cdot (m_i/m^*)^{-b} \quad (5)$$

where a_p is the coefficient for biomass production.

Model parameterization

Generally, the parameter values were derived by comparing the estimated and observed rate constants and minimizing the differences. Parameter fitting was done by maximum likelihood estimation. We distinguished between rate constants for physiological processes and rate constants describing chemical uptake and elimination. Physiological rate constants were, for example, food intake, water absorption, water excretion, and production. Chemical rate constants were, for example, chemical uptake from water ($k_{X,in,w}$) or food ($k_{X,in,f}$). Different data sets were chosen for each type of rate constant, and different parameters were derived from each type. Allometric data that relate the physiological flows to species size were used to derive the transport coefficients directly (represented by a_j in Eqn. 2). The values for the allometric rate exponents b were set to 0.25 in these regressions [16], and rate constants and mass were known. Transport coefficients can therefore be calculated using Equation 7. The differences between the observed and estimated rate constants, as described in Equation 2, were then minimized by changing the values for the partial resistances ρ_{CH_2} and ρ_{H_2O}, filling in the values for the transport coefficients as derived from the physiological flows, the default values for the lipid fraction of the organism and its food, the fractions of food assimilated and allometric rate exponent b (Table 2), and case-specific values for species weight and K_{OW}. Although the approaches for deriving transport coefficients a_j and the partial resistances ρ_p are essentially identical, the parameters had to be fitted in two steps because of the mutual dependence of the values for the partial resistances ρ_p and for the transport coefficients a_j. This mutual dependence can be seen in Equation 2. Because of this mutual dependence of the parameters, three types of parameters were distinguished: the independent parameter β, whose values could be determined without knowing the values for the transport coefficients a_j and the partial resistances ρ_p; the transport coefficients α; and the partial resistances ρ.

First, the independent parameters β were determined using data from the literature. These are the lipid fractions of organism and its food, the fractions of food assimilated, and the rate exponent b. For these parameters, default values are typically applied in OMEGA. When default values are applied, the uncertainty in the mean of these parameters affects the uncertainty in BAF estimates. To account for this fact, instead of case-specific values, we used typical values and ranges based on data from the literature that we had already used for model parameterization, as well as values from Hendriks [19] (Table 2). The lipid fractions of organism and food, as well as the fractions of food assimilated, affect the estimation of the partial resistances ρ, described below, because they are used in the calculations of the chemical intake rate constants. Therefore, deriving values for the partial resistances from the chemical intake rate constants implies that the values of the lipid contents affect the values for the partial resistances. Moreover, the allometric rate exponent b also affects the values and uncertainties of the transport coefficients a_j, described below, because it is used in the allometric relations from which the transport coefficients are derived. The rate exponent b was set to a value of 0.25 [16] (Table 2).

Second, the transport coefficients a_j were derived from allometric data [16,18] (Supplemental Data, Tables S2-S4). These coefficients are for the transport of water through the organism a_w, for the transport of food through an organism a_f, and for the production of biomass a_p. Values for the transport coefficients a_j were derived by relating the measured physiological rate constants for biomass production, food intake, and water absorption and excretion to the measured masses. Because these flows are not known to be limited by resistances in water or membranes, the transport coefficients a_j can be directly related to the rate constants k_j and calculated as the coefficients of the regression. The values of these parameters affect the estimation of the third type of parameter, the partial resistances ρ_{CH_2} and ρ_{H_2O}, as described below.

Third, the partial resistances ρ_p were derived by comparing the measured and estimated chemical rate constants and minimizing the differences by maximum likelihood estimation. Data for these chemical rate constants are included in the Supplemental Data (Tables S6-S8). These constants are the partial water-layer resistances $\rho_{H_2O,w}$ and $\rho_{H_2O,f}$, as well as the partial lipid-layer resistances $\rho_{CH_2,0}$ and ρ_{CH_2}. Instead of the intake rate of a substance from food ($k_{X,in,f}$), the fraction of the substances assimilated from food ($p_{X,f}$) is often measured

Table 2. Means and coefficient of variation (CV = standard deviation/mean) of lipid fractions, fractions of food assimilated, and the rate exponent

Parameter description	symbol	Unit	Arithmetic means	Coefficient of variation	Distribution	Source
Slope of allometric regression	b	[–]	0.25	0.11	Normal	Mean [16], CV [19]
Fraction food assimilated trophic level 2	$p_{f,2}$	[–]	0.64	0.26	Logit	Calculated from [54]
Fraction food assimilated trophic level 3	p_f	[–]	0.73	0.24	Logit	Calculated from [54]
Lipid fraction trophic level 1	$p_{CH_2,l}$	[–]	0.01	1	Logit	Calculated from [37,38,16,17], and references therein
Lipid fraction trophic level 2	$p_{CH_2,2}$	[–]	0.03	1	Logit	Calculated from [37,38,16,17], and references therein
Lipid fraction trophic level 3	$p_{CH_2,3}$	[–]	0.05	1	Logit	Calculated from [37,38,16,17], and references therein

and therefore used for calibration of the partial resistances. This substance fraction assimilated ($p_{X,f}$) can be calculated as the ratio between the rate constant for intake of the substance ($k_{X,in,f}$) and the rate constant for intake of food

$$p_{X,f} = \frac{p_f}{(1-p_f)\cdot a_f}\cdot\frac{1}{p_{CH_2,food}\cdot(Kow_x-1)+1} \times \frac{1}{\rho_{H_2O,f}+\frac{\rho_{CH_2}}{Kow_x}+\frac{1}{p_{CH_2,food}\cdot Kow_x\cdot(1-p_f)\cdot a_f}} \quad (6)$$

In addition to the sets of parameters described above, the calculation of the chemical intake and elimination rate constants requires the mass of the organism m_i and the K_{OW} value of the substance. These input variables are determined case specifically and depend on the species and substance considered.

Uncertainty analysis

Independent parameters β. The averages and variances for the lipid fractions, the fractions of food assimilated, and the allometric rate exponent b were taken from the literature, as summarized in Table 2. Logit distributions were selected for the fractions of food assimilated and the lipid fractions to ensure that the fractions were between 0 and 1, consistent with distributions for proportions [20]. Viewing $\boldsymbol{\beta}$ as a vector consisting of all (transformed) independent parameters, namely the lipid fractions, the fractions of food assimilated, and the rate exponent b, the variances can be summarized in the variance-covariance matrix $\Sigma_{\boldsymbol{\beta}}$. For the rate exponent b only, $\Sigma_{\boldsymbol{b}}$ actually represents a scalar, the variance in b.

Transport coefficients a

Allometric data to derive the transport coefficients for water absorption—excretion a_w, food intake a_f, and production a_p—were collected from the literature to represent the three levels of the aquatic food chain. Because the calculation of the concentration in fish also depends on the concentration in the invertebrates that are considered their food, rate constants for invertebrates were also included. All data available for species classes were included to form one data set for each of the physiological flows: production, water absorption, and food ingestion. Where several rate constants per species were available, the geometric means were used. Data from different sources were weighted equally. In total, 178 pairs of masses and corresponding rate constants for invertebrates, fish, amphibians, and reptiles were included [21–35] (Supplemental Data, Tables S2–4). From these allometric data, the mean values of the logarithms of the coefficients a_j were calculated as

$$\ln(a_j) = \frac{1}{n}\cdot\sum(k_j + b\cdot\ln(m_i/m^*)) \quad (7)$$

using a value of 0.25 for b [[16],Table 2], and σ, the standard error in the mean; $\ln(a_j)$, was calculated as

$$\sigma_{\ln(a_j)} = \sqrt{\frac{1}{n}\cdot\frac{1}{n-1}}\cdot\sum(\ln(k_j) + b\cdot\ln(m_i/m^*) - \ln(a_j))^2 \quad (8)$$

where n represents the number of data points in each allometric data set and k_j represents any of the physiological flows, water absorption and excretion, food intake, and production. The standard error of the mean in $\ln(a_j)$, calculated according to Equation 8, specifically reflects the uncertainty in $\ln(a_j)$ caused by the estimation of this coefficient from the allometric relations.

Viewing $\boldsymbol{\alpha}$ as a vector consisting of all $\ln(a_j)$—that is, $\ln(a_w)$ (coefficient for the transport of water through the organism), $\ln(a_f)$ (coefficient for transport of food through an organism), and $\ln(a_p)$ (the coefficient for production of biomass)—the variances calculated in Equation 8 can be summarized in the variance-covariance matrix $\Sigma_{\boldsymbol{\alpha}}$. This matrix represents the uncertainty in $\boldsymbol{\alpha}$ at constant $\boldsymbol{b}$. The uncertainty in $\boldsymbol{\alpha}$ due to the uncertainty in $\boldsymbol{b}$ can be derived by defining the random vector $(\boldsymbol{D_b\alpha})\Sigma_{\boldsymbol{b}}^{1/2}\boldsymbol{Z_b}$, where $\boldsymbol{D_b\alpha}$ is the derivate of $\boldsymbol{\alpha}$ to $\boldsymbol{b}$ from the allometric relation, and $\Sigma_{\boldsymbol{b}}^{1/2}\boldsymbol{Z_b}$ represents the random error in $\boldsymbol{b}$. The uncertainty in $\boldsymbol{\alpha}$ due to uncertainty in $\boldsymbol{b}$ can then be calculated as the variance-covariance matrix of this vector:

$$\sum\nolimits_{\alpha(\boldsymbol{b})} = (\boldsymbol{D_b}\alpha)\sum\nolimits_{\boldsymbol{b}}(\boldsymbol{D_b}\alpha)' \quad (9)$$

The total variance in $\boldsymbol{\alpha}$ due to the uncertainty in $\boldsymbol{b}$ and in $\boldsymbol{\alpha}$ itself can then be calculated as the sum of the two variance-covariance matrices:

$$\sum\nolimits_{\text{total},\boldsymbol{a}} = \sum\nolimits_{\boldsymbol{a}} + \sum\nolimits_{\boldsymbol{a}(\boldsymbol{b})} \quad (10)$$

Partial resistances ρ

For substances with K_{OW} values between 10^3 and 10^9, previously published measured data on the chemical rate constants for intake from water ($k_{X,in,w}$) by algae, invertebrates, and fish, and on the fractions of substance assimilated from food ($p_{X,f}$) by aquatic invertebrates and fish, were selected from the literature [[36–38] and references therein]. These values were compared to calculated values for the rate constants of chemical intake from water and the fractions of substance assimilated from food to estimate the most likely values for the partial resistances $\rho_{H_2O,w}$, $\rho_{H_2O,f}$, $\rho_{CH_2,0}$, and ρ_{CH_2}. For $k_{X,in,w}^{obs}$ and $p_{X,f}^{obs}$, the likelihood function L can be defined as

$$\begin{aligned} &L(\rho_{H_2O,w}, \rho_{CH_2,0}, \rho_{H_2O,f}, \rho_{CH_2}) \\ &= \prod_{i=1}^{n_k}\frac{1}{\sigma_K\sqrt{2\pi^e}}^{-0.5\cdot\left[\frac{\ln\left(k_{X,in,w}^{obs}\right)-\ln\left(k_{X,in,w}^{est}\right)}{\sigma_k}\right]^2} \\ &+ \prod_{i=1}^{n_p}\frac{1}{\sigma_p\sqrt{2\pi^e}}^{-0.5\cdot\left[\frac{\text{logit}\left(p_{X,f}^{obs}\right)-\text{logit}\left(p_{X,f}^{est}\right)}{\sigma_p}\right]^2} \end{aligned} \quad (11)$$

where n_k is the number of observations for the chemical intake rate constant from water, n_p is the number of observations for the fraction of chemical assimilated from food ($p_{X,f}$), and σ is the error in the normal distribution. The normal distribution for the logarithm of the rate constant for chemical intake from water $\ln(k_{X,in,w}^{obs})$ is characterized by the expected value $\ln(k_{X,in,w}^{est})$ and the standard deviation σ_k. The fraction of the substance assimilated from food is described by a normal distribution of $\text{logit}(p_{x,f}^{obs})$ with expected value $\text{logit}(p_{x,f}^{est})$ and standard deviation σ_p. Values for the partial resistances ρ_p were found by maximizing the likelihood function L. The partial resistances are used in the calculations of the rate constants for chemical uptake from water ($k_{X,in,w}$) and food ($k_{X,in,f}$) for several trophic levels (Eqns. 2–4,6). Therefore, all equations for partial resistances were solved simultaneously; otherwise, it would have been required to fix some partial resistances at an arbitrary value. By deriving the cross-derivates for every pair of parameters of the α, β, and ρ groups, the Fisher information matrix ($\boldsymbol{F}$) was found from the maximum likelihood estimation.

The inverse of the Fisher information matrix for $\rho_{CH_2,0}$, ρ_{CH_2}, $\rho_{H_2O,w}$, and $\rho_{H_2O,f}$ gives the variance-covariance matrix $\Sigma\rho$, which represents the uncertainty in the $\rho_{CH_2,1}$, $\rho_{CH_2,2}$, $\rho_{H_2O,w}$, and $\rho_{CH_2,2}$ resulting from the maximum likelihood estimation with constants a_j, b, lipid fractions, and fractions of food assimilated.

A total variance-covariance matrix was derived by adding the variance-covariance matrices reflecting the uncertainty in ρ and the uncertainty in ρ caused by the uncertainty in $\boldsymbol{\alpha}$ and $\boldsymbol{\beta}$.

$$\sum_{\text{total},\rho} = \sum_{\rho} + \sum_{\rho(\boldsymbol{a})} + \sum_{\rho(a,b)} \tag{12}$$

where $\Sigma\rho$ is the variance-covariance matrix of ρ as found from the maximum likelihood estimation, $\Sigma\rho_{(\boldsymbol{\alpha})}$ is the uncertainty in ρ caused by the uncertainty in $\boldsymbol{\alpha}$ calculated as the variance-covariance matrix of the random vector $(\boldsymbol{D}_{\boldsymbol{\alpha}}\rho)\Sigma_{\boldsymbol{\alpha}}^{1/2}\boldsymbol{Z}_{\boldsymbol{\alpha}}$,

$$\sum_{\rho(\alpha)} = (\boldsymbol{D}_{\alpha}\rho)\sum_{\alpha}(\boldsymbol{D}_{\alpha}\rho)' \tag{13}$$

and $\Sigma\rho_{(\boldsymbol{\alpha},\boldsymbol{\beta})}$ is the uncertainty in ρ caused by the uncertainty in $\boldsymbol{\alpha}$ and $\boldsymbol{\beta}$, calculated as the variance-covariance matrix of the random vector $((D_{\alpha}\rho)(D_b\alpha) + D_b\rho)\Sigma_b^{1/2}Z_b$,

$$\begin{aligned}\sum_{\rho(\alpha,\beta)} &= ((\boldsymbol{D}_{\alpha}\rho)(\boldsymbol{D}_{\boldsymbol{b}}\alpha) + \boldsymbol{D}_{\beta}\rho)\\ &\sum_{\beta}((\boldsymbol{D}_{\alpha}\rho)(\boldsymbol{D}_{\beta}\rho) + \boldsymbol{D}_{\beta}\rho)\end{aligned} \tag{14}$$

$D_{\boldsymbol{\alpha}}\rho$ is the derivate of ρ to $\boldsymbol{\alpha}$ from the maximum likelihood equation; $D_{\boldsymbol{\beta}}\rho$ is the derivate of ρ to $\boldsymbol{\beta}$, $\Sigma_{\boldsymbol{\alpha}}^{1/2}\boldsymbol{Z}_{\boldsymbol{\alpha}}$ and represents the random error in $\boldsymbol{\alpha}$ (square root of the variance-covariance matrix Σ times a multivariate standard normal distribution $\boldsymbol{Z}$); and $D_b\boldsymbol{\alpha}$ is the derivate of $\boldsymbol{\alpha}$ to $\boldsymbol{b}$ from the allometric relation $\Sigma_b^{1/2}\boldsymbol{Z}_b$ and represents the random error in b as in Equation 9. The approximations of Equations 9 and 15 are derived from a Taylor expansion of the uncertainties in estimated parameters, where only the first-order terms are kept. This yields a tractable application of maximum likelihood estimation. The underlying assumption is that $\Sigma_{\boldsymbol{\alpha}}$, $\Sigma_{\boldsymbol{b}}$, and the product of their square roots are small compared to the first-order terms. We consider this assumption justified because the variances are relatively small compared to the parameter values, making it unlikely that the contribution of the second- and higher-order terms would be larger than that of the first-order term. From the total variance-covariance matrix, the correlation matrix was calculated. The correlation matrix is given in the Supplemental Data (Table S5).

Probabilistic simulations

Uncertainty in estimated BAF and rate constants for fish were simulated using Monte Carlo analysis. Monte Carlo simulations were performed with Crystal Ball [39] using Latin hypercube sampling. Each simulation consisted of 100,000 trials. The fifth, 50th, and 95th percentiles of estimated BAF and rate constants for fish were generated as output. The most likely values, variances, and correlations that were derived from the literature, the allometric data, and the maximum likelihood estimations were used as input for Monte Carlo simulations. For the lipid fractions, the fractions food of assimilated, and the rate exponent b, Table 2 describes the means, variances, and distributions. For the transport coefficients and partial resistances, log-normal distributions were used, consistent with the maximum likelihood method. Their means and values are given in Table 3.

An additional analysis was performed to identify the parameters that contribute most to variation in BAF. This analysis consisted of a Monte Carlo simulation in combination with a rank correlation (expressed as percentage of total variance). For this purpose, Crystal Ball calculates the Spearman rank correlation coefficients between the output variable and each input variable. The squares of these correlation coefficients were summed, and the contribution of each input variable was derived by dividing its individual coefficient by this total sum. Multiplying these individual contributions by 100 yielded percentages representing the relative contributions of the input variables to the variance in the BAF or in the rate constant. The contribution to variance is a combination of the model's sensitivity to a parameter and the uncertainty range of the parameter.

Comparison between estimates and observations

Estimated BAFs were compared to observed BAFs for substances with K_{OW} between 10^3 and 10^7 in fish, as published earlier, and estimated and observed total elimination rates ($k_{X,out}$) for fish were compared ([2,16,37,38] and references therein). Observed BAFs were recalculated to truly dissolved concentrations assuming equilibrium partitioning, using organic-carbon partitioning coefficients from EPI Suite [40] and organic carbon fractions as reported in [2,16,37,38] and references therein. Estimates and observations were also compared for the rate constants for chemical intake from water $k_{X,in,w}$ and the fractions of substance assimilated from food $p_{X,f}$ (Supplemental Data, Tables S6-S8).

RESULTS

The estimated and observed rate constants of fish for chemical intake from water ($k_{X,in,w}$), the fractions of substance assimilated from food ($p_{X,f}$), and the total chemical elimination rate constants ($k_{X,out}$) are shown in Figure 1. Approximately 90% of the estimates were within a factor of 10 of the measured values (Fig. 1). More specifically, approximately 90% of the estimates for the fraction of substance assimilated from food and total chemical elimination rate were within a factor of 3 of the measurements. For the chemical intake rate from water,

Table 3. Maximum likelihood estimations (MLE) and 95% confidence interval (95p CI) ranges of resistances and transport coefficients determined by maximum likelihood estimation and used in the Monte Carlo simulation, and values and ranges of resistances and transport coefficients from Hendriks et al. [16].

Symbol	Description	Unit	MLE	95p CI	Values[a]	95p CI[a]
a_p	Biomass (re)production coefficient	kg· kg^{-1}·d^{-1}· kg^b	$1.1\cdot10^{-3}$	$8.1\cdot10^{-4}$–$1.5\cdot10^{-3}$	$6\cdot10^{-4}$	Not given
a_w	Water absorption-excretion coefficient	kg· kg^{-1}·d^{-1}· kg^b	$4.2\cdot10^{3}$	$2.2\cdot10^{2}$–$8.7\cdot10^{4}$	200	Not given
a_f	Food ingestion coefficient	kg· kg^{-1}·d^{-1}· kg^b	$3.0\cdot10^{-2}$	$2.2\cdot10^{-2}$–$4.1\cdot10^{-2}$	$5\cdot10^{-3}$	Not given
$\rho_{H_2O,w}$	Partial water-layer resistance to and from water	d·kg	$6.8\cdot10^{-3}$	$3.7\cdot10^{-3}$–$1.3\cdot10^{-2}$	$2.8\cdot10^{-3}$	$1.41\cdot10^{-3}$–$4.1\cdot10^{-3}$
$\rho_{H_2O,f}$	Partial water-layer resistance to and from food	d·kg	$2.0\cdot10^{-4}$	$3.6\cdot10^{-6}$–$1.1\cdot10^{-2}$	$1.1\cdot10^{-5}$	$0.0\cdot10^{-5}$–$3.9\cdot10^{-5}$
$\rho_{CH_2,0}$	Partial lipid-layer resistance, trophic level 1 (plants)	d·kg	7.8·103	$5.0\cdot10^{2}$–$1.2\cdot10^{5}$	$4.6\cdot10^{3}$	$1.3\cdot10^{3}$–$7.8\cdot10^{3}$
ρ_{CH_2}	Partial lipid-layer resistance, trophic level 2 and 3 (animals)	d·kg	97	32–298	68	30–110

[a] Hendricks et al. [16].

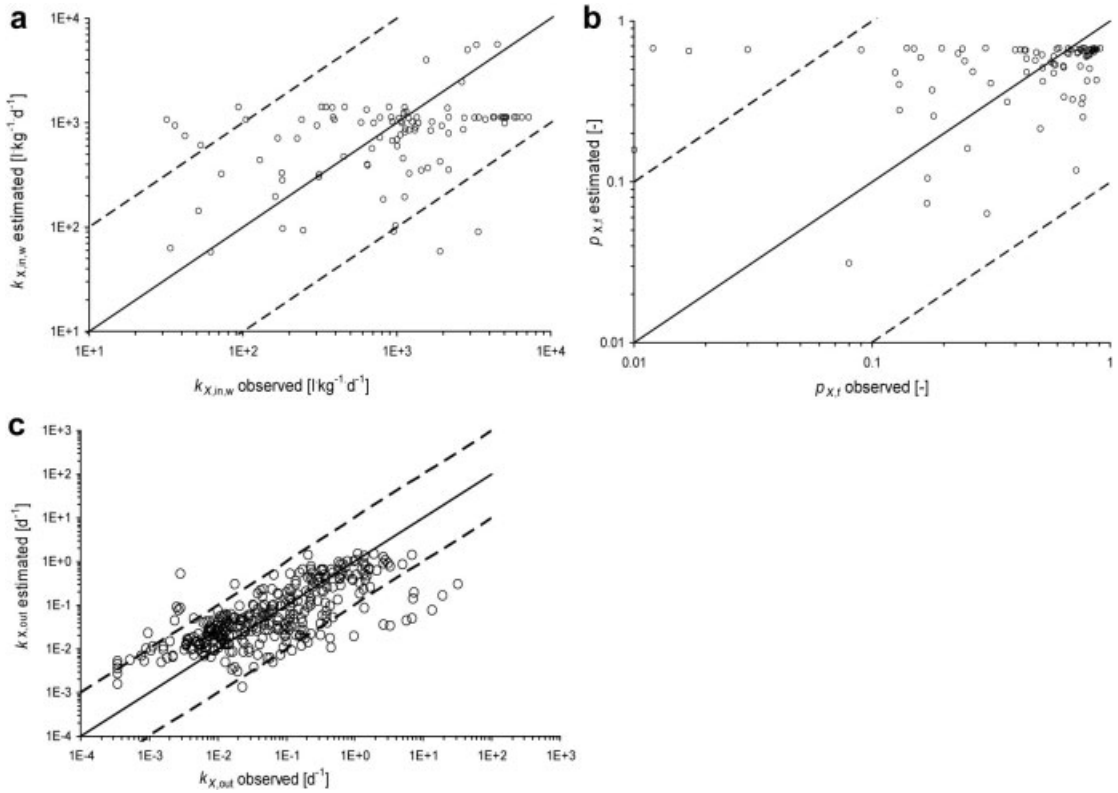

Fig. 1. Estimated versus observed rate constants for intake from water $k_{X,\mathrm{in,w}}$ [L·kg^{-1}·d^{-1}] (**a**) fractions of substance assimilated from food $p_{X,\mathrm{f}}$ [-] (**b**) and total elimination rate constant $k_{X,\mathrm{out}}$ [d^{-1}] (**c**) for fish. Solid lines indicate a one-to-one agreement between estimated and observed values, dashed lines a deviation of a factor of 10 between estimated and observed values.

approximately 50% were within a factor of 3 of the measurements.

Figure 2 shows the calculated BAFs in comparison with measured BAFs for fish. The BAFs are given in μg·kg^{-1} wet weight/μg·l^{-1} (based on dissolved concentrations in water). Uncertainty in the 50th percentile estimation represents the uncertainty in the BAF arising from the parameters taken into account in the present study. Eighty percent of the observed BAFs were within the fifth and 95th percentile of the independent estimations. The ratio between the 95th and the fifth percentile of estimations was approximately a factor of 8 to 40. The 50th percentile of the BAF estimates increased with increasing K_{OW} up to a K_{OW} value of approximately10^7 and then decreased with increasing K_{OW}.

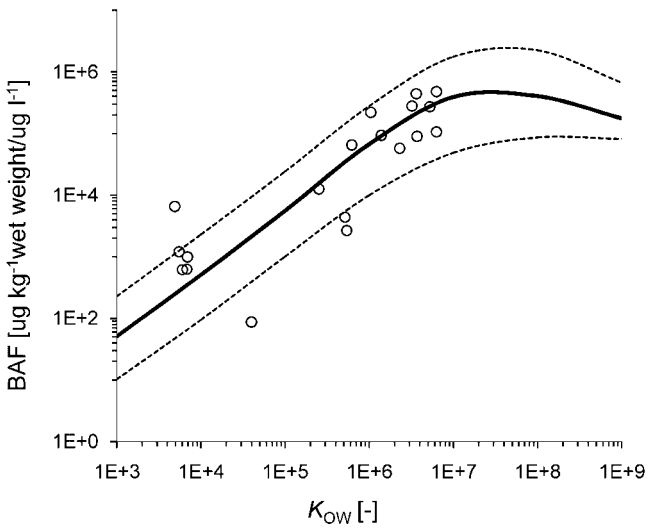

Fig. 2. Estimated (lines) and measured (circles) bioaccumulation factors [μg·kg^{-1} wet wt / μg·L^{-1} dissolved] in fish versus K_{OW}: solid line: 50th percentile of estimates, dashed lines: 90th percentile confidence interval of estimates.

Figure 3 shows the fifth, 50th, and 95th percentiles of estimates of the rate constants for chemical intake from water ($k_{X,\mathrm{in,w}}$), chemical intake from food ($k_{X,\mathrm{in,f}}$), and total chemical elimination ($k_{X,\mathrm{out}}$) for a fish of 0.1 kg. The ratio between the 95th and the fifth percentile for the chemical intake from water is always below 10 and decreases slightly between K_{OW} values of 10^3 and 10^5. The ratio between the 95th and the fifth percentile for the chemical intake rate from food increases from a factor of 3 at low K_{OW} values up to approximately a factor of 50 for high K_{OW} values. For the total chemical elimination rate constant, the ratio between the 95th and the fifth percentile decreases with increasing K_{OW}, from a factor of about 35 to a factor of approximately 5.

The results of the contribution to variance analysis are shown in Table 4. The uncertainty in partial lipid layer resistance contributed the most to the variation in the rate constants for chemical intake from water ($k_{X,\mathrm{in,w}}$) for low K_{OW} values (10^3 to 10^4); for higher K_{OW}, the partial water-layer resistances from and to water also contributed to the variation in chemical intake rates by water. The uncertainty in the fraction of food assimilated contributed the most to the variation in the rate of chemical intake from food ($k_{X,\mathrm{in,f}}$) up to a K_{OW} value of approximately 10^5; for higher K_{OW} the water-layer resistance from and to food also contributed to uncertainty in the rate of chemical intake from food. The highest variation in the total elimination rate constant ($k_{X,\mathrm{out}}$) and the predicted BAFs was caused by the variation in lipid fractions, but their influence

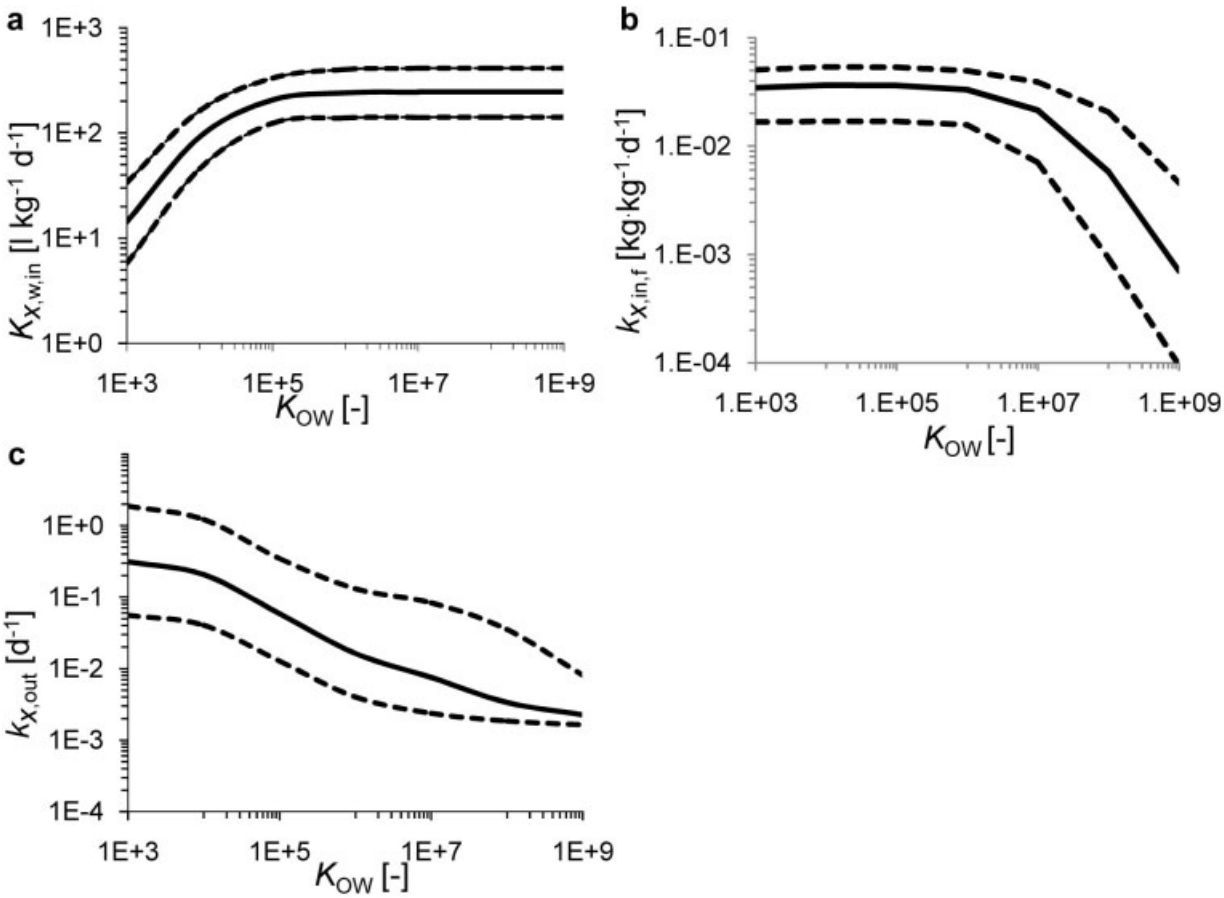

Fig. 3. The 50th (solid lines), 90th percentile confidence interval of estimates for chemical intake rate constants from water [L·kg^{-1}·d^{-1}] (**a**), chemical intake rate constants from food [kg·kg^{-1}·d^{-1}] (**b**) and total elimination rate constants [d^{-1}] (**c**) for a fish of 0.1 kg.

decreased with increasing K_{OW}. For the high K_{OW} values, the partial resistances in the water layer to and from food also contributed to the uncertainty in the estimated total elimination rate constants. For the estimated BAFs, the fractions of food assimilated also contributed to the uncertainty in the BAF for high K_{OW} values.

DISCUSSION

Parameterization

Two aspects of model parameterization are discussed: values for partial resistances and transport coefficients are compared to values found by Hendriks et al. [16], and model predictions based on these parameter values are compared to measurements for chemical intake rates from water ($k_{X,in,w}$) and the fractions of the substance assimilated from food ($p_{X,f}$), total chemical elimination rates ($k_{X,out}$), and bioaccumulation factors for fish (BAF). The observed rate constants and fractions of substance assimilated from food were a subselection of fish from the observed rate constants and fractions of substance assimilated, which were used for estimating the partial resistances and included several trophic levels; they are compared to estimated chemical rate constants for fish in Figure 1 to show how the chemical rate constants calculated from the fitted parameters approximate the observed rate constants.

The values of transport coefficients and partial resistances found by Hendriks et al. [16] are included in the last two columns of Table 3 for comparison with the values from our model parameterization. Our uncertainty ranges are generally larger than the ones found by Hendriks et al. [16]. All of our maximum likelihood estimates are comparable to the higher values found by Hendriks et al. [16] or even higher. Most values are within a factor of 10 from the values found by Hendriks et al. [16], with exception of the values for the water absorption-excretion coefficient a_w and for the partial water-layer resistance from food $\rho_{H_2O,f}$ deviate by more than a factor of 10 (a factor of 21 and 18 respectively). New data pairs of rate constants and species weight were gathered mostly for the allometric relation for water absorption-excretion. On the other hand, allometric data for the other physiological flows were also included in the parameterization by Hendriks et al. [16]. One fast rate constant in our data set explains the deviation in values for the transport coefficient a_w (Supplemental Data, Table S3).

The observed chemical intake rate constants from water ($k_{X,in,w}$) deviate from the estimated rate constants by more than a factor of 10 for chlorodibenzo-*p*-dioxins, phenols, alcohol ethoxylates, halobiocides, halophenols, phosphates, polycyclic heteroaromatic hydrocarbons, and nitrogenbiocides. A deviation between the estimated and observed values by more than a factor of 5 was also observed by Hendriks et al. [16] for chlorodibenzo-*p*-dioxins, polycyclic heteroaromatic hydrocarbons, and alcohol ethoxylates. They explained the deviation by the fact that, for labile or large molecules, the measured uptake would be less than estimated by the model. Labile substances could be ionized or metabolized, which would lead to an apparent lower observed chemical intake rate from water. Both processes are currently not included in the model. Overestimation of bioavailability could also play a role, but this possibility

Table 4. Contribution to variance from the Monte Carlo simulation in estimated chemical intake rates via water ($k_{X,\mathrm{in,w}}$), chemical intake rates via food ($k_{X,\mathrm{f,in,f}}$), and total chemical elimination rate constants ($k_{,X,\mathrm{out}}$) for a fish of 0.1 kg and bioaccumulation factors (BAFs) caused by different groups of parameters for six K_{OW} values. Numbers printed in italics indicate a contribution to variance of more than 60%.

K_{OW}	◦	10^3	10^4	10^5	10^6	10^7	10^8
		%	%	%	%	%	%
(a) $k_{X,\mathrm{in,w}}$	$\rho_{H_2O,w}$	0.1	9.4	*80.4*	*89.3*	*89.5*	*89.5*
	$\rho_{H_2O,f}$	0.0	0.0	0.0	0.0	0.0	0.0
	ρ_{CH_2}	*99.8*	*88.8*	9.7	0.1	0.0	0.0
	a_w	0.0	1.7	9.9	10.5	10.5	10.5
	b	0.0	0.0	0.0	0.0	0.0	0.0
(b) $k_{X,\mathrm{in,f}}$	$\rho_{H_2O,w}$	0.0	0.0	0.0	0.0	0.0	0.0
	$\rho_{H_2O,f}$	0.0	0.0	1.5	17.2	51.6	*67.6*
	ρ_{CH_2}	0.6	0.5	0.4	0.2	0.0	0.0
	a_f	24.7	24.3	21.7	12.8	2.6	0.2
	b	0.0	0.0	0.0	0.0	0.0	0.0
	$p_{CH_{2,2}}$	0.1	1.3	2.9	7.7	13.4	15.2
	p_f	*74.7*	*73.8*	*73.4*	*62.1*	32.4	17.0
(c) $k_{X,\mathrm{out}}$	$\rho_{H_2O,w}$	0.9	3.4	1.3	0.1	0.0	0.0
	$\rho_{H_2O,f}$	0.0	0.0	0.0	1.9	23.3	58.1
	ρ_{CH_2}	21.3	9.3	0.6	0.0	0.0	0.0
	a_p	0.0	0.0	0.0	0.0	0.6	5.0
	a_f	0.0	0.0	0.1	0.4	0.4	0.1
	a_w	0.0	0.2	0.5	0.2	0.0	0.0
	B	0.0	0.0	0.0	0.0	0.0	0.0
	$p_{CH_{2,3}}$	*78.5*	*89.2*	*92.1*	*76.6*	*55.7*	32.5
	$p_{CH_{2,3}}$	0.1	0.3	2.4	14.2	14.1	3.0
	p_f	0.0	0.1	0.8	5.4	5.8	1.3
(d) BAF	$\rho_{H_2O,w}$	0.0	0.0	0.1	0.0	1.3	5.0
	$\rho_{H_2O,f}$	0.0	0.0	0.0	0.4	8.5	34.7
	ρ_{CH_2}	0.0	0.0	0.0	0.4	0.4	0.0
	$\rho_{CH_{2,0}}$	0.1	0.0	0.0	0.0	0.0	0.0
	a_p	0.0	0.0	0.0	0.3	1.8	3.5
	a_f	0.0	0.0	0.1	0.2	0.2	0.1
	a_w	0.0	0.0	0.0	0.0	0.2	0.7
	b	0.0	0.0	0.0	0.0	0.0	0.0
	$p_{CH_{2,3}}$	*99.5*	*99.1*	*94.0*	*69.4*	35.4	11.3
	$p_{CH_{2,2}}$	0.2	0.4	1.8	1.4	0.6	4.7
	$p_{CH_{2,1}}$	0.0	0.0	0.0	0.0	2.4	8.7
	p_f	0.2	0.5	3.9	23.8	36.0	23.7
	$p_{f,2}$	0.0	0.0	0.1	4.0	13.1	7.8

$p_{CH_{2,1}}$, Lipid fraction trophic level 1; $p_{CH_{2,2}}$, Lipid fraction trophic level 2; $p_{CH_{2,3}}$, Lipid fraction trophic level 3.

is considered less relevant in the present study because BAFs are compared using dissolved concentrations.

The fractions of substances assimilated from food ($p_{X,f}$) were overestimated by more than a factor of 10 for a few substances from the halobenzenes, chlorodibenzo-*p*-furans, and halobiphenyls. This overestimation could be related to an underestimation of permeation resistances for large molecules, an overestimation of the food intake, or disregard of metabolic transformation of chemicals in the organisms (e.g., [38,41]).

The majority (60%) of the estimated total chemical elimination rates ($k_{X,\mathrm{out}}$) in Figure 1c that deviate more than a factor of 10 from the observed elimination rates are lower than the observed values. The underestimation of observed total chemical elimination rate constants is generally explained by metabolic transformation in the organism [16,42]. Hendriks et al. [16] report possible metabolic transformation for polycyclic heteroaromatic hydrocarbons, phosphorbiocides, phenols, nitrogenbiocides, halobiphenyls, halobiocides, haloaliphatic hydrocarbons, chlorodibenzo-*p*-furans, chlorodibenzo-*p*-dioxins, and alcohol ethoxylates. The elimination of these substances is underestimated in Figure 1. These substances partly overlap with the substances for which the measured uptake rates were lower than the estimated rates. This finding suggests a common mechanism, such as a fast metabolism, that leads to deviation between observations and estimations.

The decrease in BAF for chemicals with K_{OW} higher than 10^6–10^7 (Fig. 2) is expected from model predictions [6], because chemical uptake and elimination rates generally decrease with increasing K_{OW}, with the exception of concentration dilution through production of biomass ($k_{\mathrm{out},p}$). This decrease in rate constants leads to a constant minimum elimination that is independent of the substance, therefore leading to an overall decrease in BAF at K_{OW} values of 10^6–10^7. Because all BAF measurements in Figure 2 are for chemicals with K_{OW} values lower than 10^7, the predicted decrease in BAF in the high K_{OW} range could not be validated by measurements.

Uncertainty ranges

Model applications generally distinguish different types of uncertainty: scenario uncertainty, model uncertainty, and parameter uncertainty [43]. Of these types of uncertainties, the biota-related parameter uncertainty in calculating BAF for fish was assessed in the present study. Because metabolic transformation is not generally considered in the model, uncertainty in metabolism was not taken into account. The findings are therefore primarily applicable for persistent organic chemicals. Additionally, BAFs were expressed on dissolved concentrations. Keeping these limitations in mind, our results can be compared to results from other studies dealing with uncertainty in BAF estimates.

Watanabe et al. [9] adjusted rate constants from the literature based on concentration measurements using Markov chain Monte Carlo simulation. They report uncertainty in the rate constants of about one to two orders of magnitude (95th and fifth percentile range), which is comparable to our uncertainty ratios. For higher K_{OW} 90% confidence interval lines are not parallel anymore (Fig. 3c). The uncertainty in the total chemical elimination rate constants ($k_{X,\mathrm{out}}$) is composed of the uncertainty in elimination by water ($k_{X,\mathrm{out,w}}$), feces ($k_{X,\mathrm{out,f}}$) and dilution by production of biomass ($k_{\mathrm{out},p}$). The production of biomass represents a lower bound of the total elimination rate constant, because calculated reduction of chemical concentration will be at this level if rate constants of elimination decrease. With increasing K_{OW} values, the rates of elimination by water and feces decrease, whereas the dilution in biomass is independent of K_{OW}. Therefore, for K_{OW} values of about 10^6, the fifth percentile is about the same as the concentration dilution by production of biomass and does not decrease any further, whereas in the larger total elimination rates elimination by water and feces still play a role, leading to decreases in the 50th and 95th percentile of the total elimination rate constant and hence to uncertainty lines that are no longer parallel. Burkhard [44] assessed the uncertainty in BAF estimates arising mainly from K_{OW}, lipid fractions, species weight and food preferences. The reported uncertainty is is four times smaller than our uncertainty estimate, which is probably due to a 20 times smaller coefficient of variation for lipid fractions than ours in Table 2. De Laender et al. [10] included chemical parameter uncertainty in their analysis, but included lower variation in lipid contents for fish than in the present study. Their reported ratio of the 90th and 10th percentiles for internal concentrations was between 10 and 30, which was largely caused by uncertainty in K_{OW}, and is comparable to our results.

Other authors also report the relevance of uncertainty in K_{OW} for uncertainty ranges of BAFs [10,13–14,45]. We considered K_{OW}, a case specific input parameter that had no influence on model parameterization. However, the total uncertainty in BAF

estimations will be higher than the ranges given in the present study. As an example, we performed additional calculations for benzo[*a*]anthracene, for which the details are given in the Supplemental Data (Table S14). Including the uncertainty in BAF estimations due to the uncertainty in the K_{OW} value of benzo[*a*]anthracene, as derived from Mackay et al. [46], led to an increase in the 95th and fifth percentile ratio of about a factor of 3.

Several authors also report that bioaccumulation in fish might depend on metabolic transformation (e.g., [47,48]). The uncertainty ranges for nonpersistent substances could therefore be different than assessed in the present study without metabolic transformation. We investigated this effect by including metabolic transformation rates for benzo[*a*]anthracene in fish and invertebrates as reported by Moermond et al. [47], taking uncertainty factors from Arnot et al. [42] for the case of the measured total elimination rate constants (Supplemental Data, Table S14). The uncertainty in estimated BAFs decreases with a factor of 5 when the uncertainty in metabolic transformation is included, whereas the estimated BAFs decrease by about a factor of 185. The estimated uncertainty decreases because metabolic transformation becomes the dominant route of elimination (up to 97%) and the uncertainty in the rate constant for metabolic transformation is in this case smaller than the uncertainty in the other parameters. These results indicate that for chemicals subject to metabolic transformation, the uncertainty in metabolic transformation rates is dominant for the uncertainty in BAF estimations and uncertainty in BAF estimations can therefore best be reduced by reducing uncertainty in metabolic transformation.

Species weight and external concentration dissolved in water were assumed to be known in the present study. Although several authors [12,14,49] reported the (potential) contribution of the uncertainty in dissolved concentrations to the uncertainty in estimation of internal concentrations, uncertainties in external concentrations are not expected to cause errors in BAF estimates, because the BAFs were expressed in terms of dissolved concentrations in the present study. The uncertainties in species weight cancel out in the calculation of the BAF. Burkhard [44] showed that varying organism weights indeed had little influence on the uncertainty ranges in the BAFs of fish.

Contribution to variance

It can be concluded from Table 4 that the uncertainty in the lipid contents and fractions of food assimilated have larger effects on the uncertainty in the estimated BAF and total chemical elimination rate constants than the uncertainties arising from transport coefficients and partial resistances. This result is in line with the findings of De Laender et al. [10]. For chemical intake rates in water, the partial resistances play a role, as they are independent of lipid contents of fish or prey and food assimilation. The calculation of the rate constants for chemical intake from water does not depend on the lipid fractions of fish or prey or the fractions of food assimilated. In calculating the variation in this rate constant, the variation in partial resistances ρ, that is, the water layer resistance to and from water and the lipid layer resistance in the organism had the greatest impact. The variation in estimated rate constants for chemical intake from water decreases with increasing K_{OW} because the lipid layer resistance decreases. A shift in the importance from the lipid-layer resistance to the water-layer resistance from a K_{OW} of approximately 10^5 has been observed by others [1,50–52].

The large contribution of the variance in lipid content of fish to the variance in estimated BAF is consistent with literature (e.g., [13,14,53]) and indicates that estimation of BAFs in fish can be improved by including case-specific lipid contents when available. The large influence of the variance in lipid content is not surprising, given the success of fish bioaccumulation models that use K_{OW} and lipid content as the only parameters [53]. The present study demonstrates that this large influence of the lipid content holds true even after considering various other descriptors to predict BAF. The influence of the uncertainty in lipid fraction of fish on the total uncertainty for the estimation of the BAF for fish can be understood from the fact that this lipid fraction is used in the calculation of the affinity ratio (Eqn. 4). The affinity ratio, which is approximately equal to $1/\left(p_{CH_2} \cdot K_{OW}\right)$ for hydrophobic substances, was only used for calculation of the elimination rate constants, whereas all other parameters were used in calculation of chemical intake and elimination rates and therefore cancelled out in the calculation of the BAF. The variation in the estimated total chemical elimination rate constants was also largely caused by the variation in lipid contents of fish because of the retention ratios. Next to the affinity ratio, the correction factor for intake from food ($1/r$, Eqn. 3) decreases with increasing K_{OW}. With increasing K_{OW} values, the influence of the chemical elimination rate by water ($k_{X,out,w}$) decreases while the influence of the variation in food assimilated on the uncertainty in the BAF estimation increases. A shift in the importance of chemical intake and elimination routes from water to food with increasing K_{OW} has been observed earlier (e.g., [6,13,14]).

The results of our research imply that assuming no metabolic transformation and BAFs calculated using truly dissolved water concentration the largest ecological parameter uncertainty in the estimation of bioaccumulation factors for fish is caused by the uncertainty in the lipid contents of the fish, up to a K_{OW} of approximately 10^6. For higher K_{OW} values, the contributions of the uncertainty in the fraction of food assimilated and the uncertainty of the water and partial lipid layer resistances to the uncertainty in the estimated BAF increases. Without measured lipid contents, the uncertainty in the estimated BAFs is approximately an order of magnitude (95th percentile/5th percentile ratio). However, a case study on benzo[*a*]anthracene showed that the uncertainty arising from this parameter might, for nonpersistent pollutants, be overshadowed by the uncertainty in metabolic transformation rates. The results imply that species-specific information on lipid content is important for the reliable estimation of accumulation of persistent organic pollutants and subsequent risk of chemicals in fish. They also imply that the uncertainty arising from the case-specific but often unknown biota-related parameters contributes more to the overall uncertainty of the BAFs of persistent organic pollutants than the uncertainty arising from the parameters that were calibrated during model development (transport coefficients and resistances).

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