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DEVELOPMENT AND EVALUATION OF A MECHANISTIC BIOCONCENTRATION MODEL FOR IONOGENIC ORGANIC CHEMICALS IN FISH

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Abstract—A mechanistic mass balance bioconcentration model is developed and parameterized for ionogenic organic chemicals (IOCs) in fish and evaluated against a compilation of empirical bioconcentration factors (BCFs). The model is subsequently applied to a set of perfluoroalkyl acids. Key aspects of model development include revised methods to estimate the chemical absorption efficiency of IOCs at the respiratory surface (E_W) and the use of distribution ratios to characterize the overall sorption capacity of the organism. Membrane–water distribution ratios (D_{MW}) are used to characterize sorption to phospholipids instead of only considering the octanol–water distribution ratio (D_{OW}). Modeled BCFs are well correlated with the observations (e.g., $r^2 = 0.68$ and 0.75 for organic acids and bases, respectively) and accurate to within a factor of three on average. Model prediction errors appear to be largely the result of uncertainties in the biotransformation rate constant (k_M) estimates and the generic approaches for estimating sorption capacity (e.g., D_{MW}). Model performance for the set of perfluoroalkyl acids considered is highly dependent on the input parameters describing hydrophobicity (i.e., log K_{OW} of the neutral form). The model applications broadly support the hypothesis that phospholipids contribute substantially to the sorption capacity of fish, particularly for compounds that exhibit a high degree of ionization at biologically relevant pH. Additional empirical data on biotransformation and sorption to phospholipids and subsequent incorporation into property estimation approaches (e.g., k_M, D_{MW}) are priorities with respect to improving model performance. Environ. Toxicol. Chem. 2013;32:115–128.
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INTRODUCTION

It was recently estimated that of the nearly 150,000 compounds preregistered under the Restriction of Chemicals regulations of the European Union (REACH), approximately 50% are acids, bases, or zwitterionics (i.e., ionogenic compounds) [1]. Categories of chemicals that have a greater tendency to be ionogenic include surfactants (anionic and cationic), pharmaceuticals (predominantly bases), and some classes of pesticides (e.g., systemic herbicides and fungicides) [1–3]. Perfluoroalkyl acids (PFAAs) such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are among the most widely known group of ionogenic organic chemicals (IOCs) due to public health concerns and the research effort dedicated to understanding the fate, transport, and bioaccumulation potential of these compounds over the past decade [4–6]. Environmental risk assessment of pharmaceuticals has also become an increasingly important issue from both a research and a regulatory perspective [7,8].

Mechanistic approaches for modeling the fate and transport of IOCs have been described in the literature and applied to various compounds (e.g., Trapp et al. [2]). These models represent an important contribution to the set of decision-support tools required to evaluate the potential human and ecological risks resulting from exposure to these chemicals. Bioaccumulation models that relate external to internal concentrations (e.g., mechanistic mass balance models, quantitative structure–property relationships) are also required for bioaccumulation assessments and to complement fate and transport calculations for exposure and risk assessment. The majority of currently available bioaccumulation models specifically developed for IOCs in fish are regression-based [9–12], although an equilibrium partitioning approach considering the role of sorption to proteins was recently proposed to characterize the bioaccumulation potential of some PFAAs [13]. Generic methods for estimating the tissue–plasma partitioning of IOCs have also been described in the literature [14,15]. Characterizing the sorption capacity of an organism (and of its diet and fecal egesta) is a central element of mechanistically based bioaccumulation models [16]. The key task is then to integrate chemical partitioning and distribution information with uptake and elimination kinetics. Advantages of mechanistic bioaccumulation models include: (1) the possibility of parameterizing them for specific organisms and exposure conditions (i.e., laboratory and field); (2) the potential to explicitly consider biotransformation on either a whole-body or tissue-specific basis; and (3) the ability to identify key uptake and elimination processes and hence the most important input parameters and uncertainties.

The present study outlines the development, parameterization, and evaluation of a mechanistic bioconcentration model for IOCs in fish. The model is based on an existing approach for neutral organic chemicals [16] but was modified to account for dissociation of IOCs. Empirical bioconcentration factors (BCFs, in L/kg) for various IOCs in fish, including some PFAAs, were compiled and evaluated for general quality.

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

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The evaluated BCF data were not used to develop the model but as an independent dataset to evaluate (test) the different model hypotheses. Research priorities to improve model performance are discussed.

THEORY

General bioaccumulation model

Bioaccumulation of organic chemicals in fish is the net result of competing processes driving uptake from the ambient environment (e.g., by gill ventilation, ingestion of contaminated food items) and elimination from the body (e.g., by gill ventilation, fecal egestion, biotransformation of parent compound). These processes have successfully been described for neutral organic chemicals using mechanistic models (e.g., based on either first-order rate constant [16] or fugacity-based approaches). For this model application, test organisms (fish) are assumed to be fed "clean" food throughout the duration of the exposure period (i.e., the concentration of chemical in diet is negligible and the related uptake term can be omitted) and only uptake from water needs to be considered. The chemical concentration in an aquatic organism (C_B, in kg/kg) over time can be modeled using the following first-order, one-compartment model

$$\frac{dC_B}{dt} = k_U C_W - k_E C_B \quad (1)$$

where k_U is the rate constant describing uptake via gill ventilation (in L/kg/d), C_W is the truly dissolved concentration of chemical in the water column (in kg/L), and k_E is the total elimination rate constant (in 1/d) characterizing the sum of all elimination processes including respiratory exchange back to the water column (k_W), fecal egestion (k_F), biotransformation (k_M), and growth dilution (k_G). Note that whereas dietary uptake can be omitted from a BCF model, fecal egestion must be included to account for redistribution of the chemical between the organism and its gut contents. At steady-state (i.e., $dC_B/dt = 0$), the concentration of chemical in the organism and the corresponding BCF (in L/kg) can be calculated by simple rearrangement

$$C_B = \frac{k_U C_W}{k_E} \text{ and } \mathrm{BCF} = \frac{C_B}{C_W} = \frac{k_U}{k_E} \quad (2)$$

Conceptually, the same processes for neutral organics apply to IOCs and it is therefore logical to adopt the mechanistic framework for neutral organics as a starting point. As outlined below, we have modified the existing Arnot and Gobas bioaccumulation model [16] for neutral organic chemicals. Readers are directed to the original publication for complete details on the development, parameterization, and evaluation of the previous model. Two fundamental changes were made in the revised model. The first is the treatment of passive uptake and elimination at the gill and the second is the treatment of the organism's sorption capacity for IOCs.

Theoretical considerations for model revisions for IOCs

Two key concepts from the pharmaceutical literature that inform the bioaccumulation potential of IOCs are the pH-partition hypothesis and the pH-piston hypothesis. The pH-partition hypothesis [17] is used to rationalize observations that IOCs that exhibit a relatively high degree of ionization cross biological membranes at a faster rate than expected based on the permeability of the membrane toward charged species (assumed to be negligible). The reason that these compounds can be absorbed efficiently is that the diffusive flux through the organic layers of the membrane is primarily (or entirely) a result of transport of the neutral rather than the charged species. Equilibrium between the neutral and charged form is then rapidly established on the other side of the membrane (see Supplemental Data, Section S1). Thus, there is no reason to presume that uptake of all IOCs across biological membranes is highly impeded. The pH-piston hypothesis [18] is used to rationalize observations that the distribution ratios characterizing sorption of IOCs to phospholipid bilayers/membranes (D_{MW}, i.e., the weighted average of the partition coefficients of the neutral and charged form) are far less sensitive to pH and the degree of ionization than would be expected based on distribution ratios in octanol–water systems (D_{OW}) or the assumption that only the neutral form of the compound interacts with the organic phase (see Supplemental Data, Section S2) [18–20]. Association of charged species with phospholipid membranes (e.g., composed of phosphatidyl cholines) is supported by electrostatic interactions with the zwitterionic head group at the interface and specific or nonspecific interactions with the rest of the molecule toward the core of the bilayer membrane. Anions can interact favorably with the choline (quaternary amine) group whereas cations can interact favorably with the phosphate group. In other words, both the neutral and charged species can interact with phospholipids. These considerations were explored by Escher et al. [19], who developed and applied a sorption model to explain these observations. Both steric and electrostatic effects are important. For example, cations are expected to interact more favorably with phospholipids than anions (related to charge positioning), and primary amines (R-NH_2) can interact more favorably than tertiary amines (R_3-N; related to "bulkiness" or steric hindrance). These two concepts and the underlying empirical data outlined above support the notion that IOCs may exhibit substantial bioaccumulation potential, particularly if they are highly persistent (i.e., not susceptible to rapid biotransformation in vivo). Irrespective of bioaccumulation potential, ecological exposure to certain groups of IOCs may be of concern because many of them are commercialized specifically because of their biological activity or toxicity (e.g., pharmaceuticals and pesticides).

Research on the sorption capacity of different lipid classes for neutral organic chemicals has revealed substantial differences in estimated partition coefficients [15,21–23]. These differences, especially the role of phospholipids, are potentially even more important for IOCs. Perfluoroalkyl acids present an interesting case study. Whereas a great deal of attention has focused on elucidating the nature and strength of PFAA interactions with serum albumin and liver fatty acid proteins [24,25] (see Supplemental Data, Section S3), several studies have also demonstrated that PFAAs interact strongly with phospholipid bilayers [26–29]. The observation that elevated wet-weight concentrations of these compounds occur in organs expected to have relatively high phospholipid contents (e.g., liver ~2% neutral lipid, 4.5% phospholipid, 15–20% protein vs muscle ~0.4% neutral lipid, 0.4% phospholipid, 20% protein) [15,30–32] is also highly suggestive. Though site-specific binding interactions (e.g., protein binding) exhibited by PFAAs may not be adequately characterized by the available approaches to describe sorption to biological macromolecules [33,34], it must also be recognized that other endogenous and exogenous ligands may have greater binding affinity and presence at binding sites, particularly when considering environmentally relevant exposure scenarios. Jones et al. [24] argued that bind-

ing to intracellular fatty acid binding proteins was unlikely to be important biologically for PFAAs due to the presence of oleic acid (with a binding affinity to liver fatty acid binding protein 500 times higher than PFOS) and other natural ligands. Site-specific binding interactions may be most relevant in the bloodstream, and its importance should not necessarily be generalized to all other tissues or the entire organism, particularly when based on in vitro data alone. For example, the in vitro protein–water distribution coefficients determined by Bischel et al. [35] using bovine serum albumin are inconsistent with available fish BCF data; PFOA is reported to have a protein–water distribution coefficient similar to that of PFOS and higher than all longer chain length perfluorinated carboxylic acids included in the study, yet it has lower reported BCFs on a blood, liver, and whole-body basis [30]. Experimental results reported by Han et al. [25] also suggest that the importance of protein binding for PFAAs is tissue specific and that these compounds can interact favorably with phospholipids (see Supplemental Data, Section S3). Minimally, the hypothesis that phospholipids play an important role in determining the bioaccumulation potential of IOCs in general and PFAAs in particular needs to be examined further.

Revisions to the existing model incorporating these theoretical considerations are described in the following sections.

Absorption

The uptake rate constant for neutral organic chemicals from the water column at the respiratory surface (k_U) is estimated in the original model [16] using the following expression

$$k_U = E_W \frac{G_V}{W_B} \quad (3)$$

where E_W is the chemical absorption efficiency across the gill membrane, G_V is the gill ventilation rate (in L/d), and W_B is the mass of the organism (kg). E_W is estimated using the following expression based on the two-resistance concept [36]

$$E_W = (R^*_W + R^*_O / K_{OW,N})^{-1} \quad (4)$$

where R^*_W and R^*_O are coefficients characterizing the relative resistances of chemical transport through multiple aqueous (water) and organic diffusion layers at and in the gill, respectively, and $K_{OW,N}$ is the octanol–water partition coefficient (neutral organic compounds) used to quantify partitioning between water and organic phases. The default values for R^*_W and R^*_O (1.85 and 155, respectively) were derived from empirical data on the uptake and elimination of neutral organic compounds in rainbow trout under defined conditions [36]. E_W is approximately 0.05 for chemicals with $\log K_{OW,N} = 1$, and passive diffusion is controlled by mass transport resistance in the organic phases (i.e., chemical concentrations in organic phases are relatively low). E_W increases with increasing $K_{OW,N}$ up to a maximum value of approximately 0.54 for chemicals with $\log K_{OW,N} \geq 4$, at which point chemical diffusion is controlled by mass transport in the aqueous layer (i.e., chemical concentrations in aqueous phases are relatively low; see Supplemental Data, Section S4).

Uptake and elimination rate constants measured for a few anionic surfactants, substituted phenols, and carboxylic acids and correlations with $K_{OW,N}$ suggest that hydrophobicity plays a key role in the uptake and elimination of IOCs [37–40]. General considerations for estimating E_W for IOCs include the following: mass transfer of the neutral form of the IOC; (2) mass transfer of the charged form of the IOC (alone or in association with a counter-ion); (3) the degree of ionization in the aqueous phases (pH-driven); and (4) the rate of re-equilibration between the neutral and charged forms. Assuming that the original E_W expression can be applied to the neutral form of IOCs and that re-equilibration between the neutral and charged forms is relatively fast compared to mass transport processes, modifications to Equation 4 are required to account for the degree of ionization (i.e., the Henderson–Hasselbalch equation) and potential transport of the charged form of the IOC.

According to Overton's rule [38,41], membrane permeation rates (i.e., transcellular passive diffusion) of neutral organic compounds are a function of the diffusivity of the chemical in the organic phase and the partition coefficient between the aqueous and organic phase, often represented by $K_{OW,N}$. Membrane permeation rates of the charged form of IOCs are expected to be orders of magnitude lower. For example, membrane permeation rates of the charged form of some anionic IOCs derived from experiments using membrane vesicles ranged from 1,000 to 30,000 times lower than those of the corresponding neutral form [38,42]. Similar studies with cationic IOCs are lacking. However, studies reporting that hydrophobic anions (e.g., tetraphenylboron) sorb to and diffuse across lipid bilayers much more efficiently than cationic analogs (e.g., tetraphenylphosphonium) [43] imply that membrane permeation rates of the charged form of organic bases are even lower relative to the neutral form compared to anionic IOCs. These findings were rationalized in terms of the positive dipole potential in the region between the aqueous phase and interior of the membrane bilayer [43,44]. Regardless of whether or not these observations are broadly representative, the charged form of both anionic and cationic IOCs can be associated with counter-ions in the aqueous phase and therefore transport as an ion pair may be a more important consideration [45,46]. An additional uptake pathway for cationic substances in association with anionic phospholipids has also been proposed and included in a theoretical passive absorption model of the human gastrointestinal tract (GIT) [47]. The relevance of this pathway for chemical absorption efficiency at the gills is unclear. However, empirical data on the bioconcentration and tissue distribution of quaternary amine surfactants (e.g., hexadecylpyridinium, CAS 140-72-7) [37,48] seem to confirm that gill membranes are permeable to organic cations (alone or as an ion pair).

As discussed above, sorption of the charged form of IOCs to membranes and subsequent translocation is influenced by both hydrophobic and electrostatic interactions (both with the membrane phospholipids and with counter-ions in the aqueous diffusion layers). Steric factors (e.g., potential for charge shielding) may also play a role [19,42]. Many uncertainties are associated with quantifying the role of these factors in determining chemical absorption efficiency. Membrane permeation rates of the charged form have been estimated from the apparent octanol–water partition coefficient of the charged form ($K_{OW,I}$) using regression-based approaches derived for neutral organic chemicals (e.g., Fu et al. [12]). Such estimations are based on Overton's rule but implicitly assume that the diffusivity of the charged form in the organic phase is similar to that of the neutral form (even though the neutral form cannot engage in the same type of electrostatic interactions). It is also important to note that $K_{OW,I}$ is typically assumed to be two to four orders of magnitude lower than $K_{OW,N}$, which, based on empirical data on octanol–water distribution ratios (D_{OW}) of IOCs as a function of ionic strength [49], largely reflects the partitioning behavior of the charged form in association with counter-ions (not the charged form itself). These observations indicate that $K_{OW,I}$ is a function of both chemical and

environmental properties and complicate the use of this parameter to estimate the membrane permeation rate or chemical absorption efficiency.

Erickson et al. [38,39] developed a sophisticated mechanistic model describing the uptake of IOCs across gill membranes and applied it to describe the gill uptake of a series of weak acids (chlorinated phenols, $pK_a = 4.74–8.62$; $\log K_{OW,N} = 2.75–5.12$) in rainbow trout. The membrane permeation rate of the charged form was assumed to be 10,000 times lower than that of the neutral form for all IOCs. The data and mechanistic gill model indicate that the presence of the ionic species in the aqueous phases supports the diffusive flux of total chemical (neutral and ionic). These considerations form the basis for the revised treatment of E_W for IOCs through the addition of two new parameters, μ and β

$$E_W = \left(R^*_W + \mu R^*_O \Big/ K_{OW,N}\right)^{-1} + \beta \quad (5)$$

The parameter μ is the ratio of the total concentration of both species to the concentration of the neutral species. For acids $\mu = 1 + 10^{(pH - pK_a)}$ and for bases $\mu = 1 + 10^{(pK_a - pH)}$ such that E_W now depends on the hydrophobicity of the neutral form, the pK_a of the chemical, and the pH at the gill. The parameter μ approaches 1.0 when the neutral species strongly dominates and becomes larger as the degree of ionization increases (i.e., fraction of the chemical in charged form increases). Essentially, μ characterizes the relatively greater resistance that dissociating chemicals incur during transport in organic phases compared to transport in aqueous phases (where the diffusive flux is supported by the presence of both neutral and charged forms).

The parameter β seeks to address two potential processes for chemical transfer at the gill that are poorly quantified. First, β is assumed to represent the potential mass transfer of the charged form through the organic layers of the gill interface (alone or in association with a counter-ion). In this context, β is most relevant for compounds exhibiting a high degree of dissociation in the aqueous phase (e.g., organic acids with $pK_a < 3$, organic bases with $pK_a > 11$). Second, β addresses the potential for passive uptake via paracellular routes (i.e., "pore transport" through tight junctions of epithelial cells) [40,50,51]. For most chemicals, the contribution of this transport pathway is expected to be low (i.e., $\beta < 0.05$) due to the relatively high transepithelial electrical resistance exhibited by gill tissues [50,51]. Given that empirical data for IOCs are limited and there are many uncertainties related to the estimation and incorporation of membrane permeation rates for charged IOCs into the E_W expression, we have assumed a generic value for β for all IOCs. Calculations were conducted assuming $\beta = 0.005$ and 0.0005 (i.e., the chemical absorption efficiency of the charged form is 10–100 times lower than a neutral compound with $\log K_{OW,N} = 1$ and 100–1,000 times lower than the maximum E_W value).

Figure 1 illustrates changes in E_W as a function of $K_{OW,N}$ and pK_a for hypothetical organic acids and bases with $\log K_{OW,N}$ from 0 to 10 and pK_a from 0 to 10 and from 3 to 13, respectively. For these calculations, it was assumed that: (1) $\beta = 0.005$, (2) the pH of the external aqueous phase is 7.0, and (3) the pH at the gill interface is one order of magnitude lower. Given these assumptions, the revised E_W can be up to two orders of magnitude lower than the original value, as can be seen for strong acids and bases with moderate hydrophobicity. The overall patterns in Figure 1 can be explained as follows. As the degree of ionization increases (i.e., $\mu\uparrow$), the organic phase resistance term increases relative to the aqueous phase resistance term, resulting in potentially lower values of E_W for dissociated IOCs compared to the original model. However, as chemical hydrophobicity increases ($K_{OW,N}$), the relatively slower transport through organic phases due to dissociation is countered to a degree by increased mass transfer to the organic phases. Note that the minimum value of the revised E_W is bounded by the value assumed for β (i.e., 0.005). As expected, the influence of the assumptions regarding β is limited across a wide range of property combinations (e.g., does not contribute substantially to the E_W for weaker acids and bases).

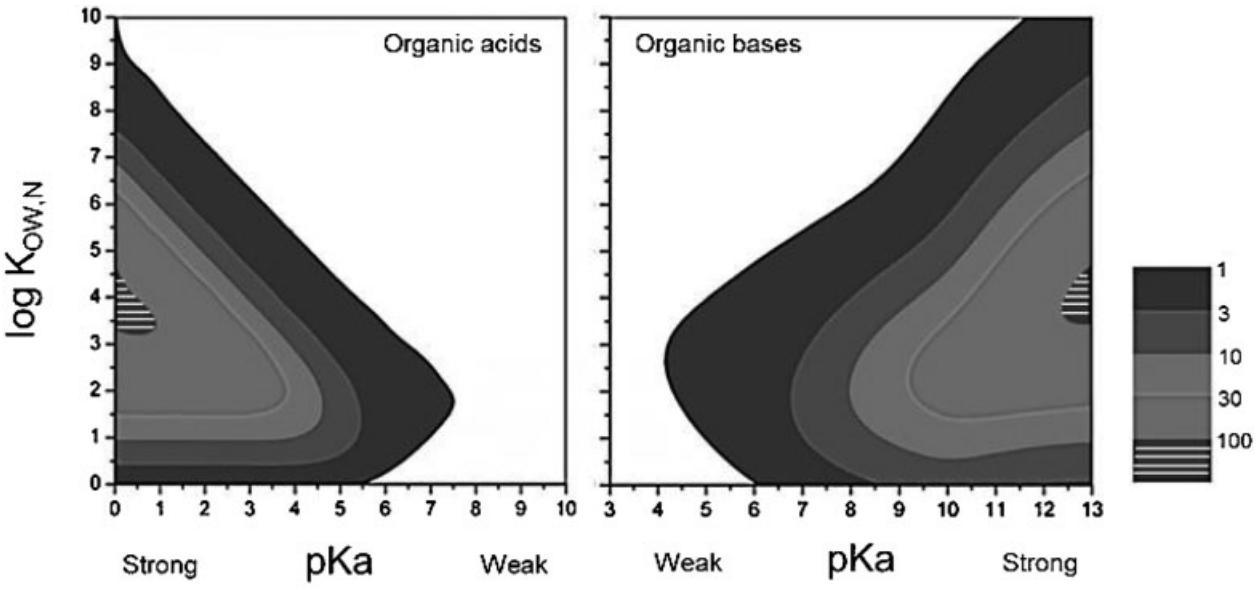

Fig. 1. Change (reduction) in the chemical uptake efficiency at the gills (E_W) on model revision (expressed as the ratio of E_Ws in the original and revised approach) as a function of the octanol-water partition coefficient of the neutral form ($\log K_{OW,N}$) and pK_a for organic acids and bases assuming a bulk water pH of 7 and pH at the gills of 6.

Sorption capacity

The sorption capacity of the organism for neutral organic chemicals compared to water is estimated in the original model [16] using a three-phase approach, represented by Equation 6 shown below

$$K_{BW} = f_L K_{OW,N} + f_{NLOM}\rho_{NLOM}K_{OW,N} + f_W \quad (6)$$

where K_{BW} (in L/kg) is the biota–water partition coefficient, f_L, f_{NLOM}, and f_W are the volume fractions of total lipid, nonlipid organic matter (i.e., proteins, carbohydrates), and water in the organism (whole-body), respectively, and ρ_{NLOM} is a proportionality constant relating the sorption capacity of NLOM to octanol. The sorption capacity of ingested food items and fecal egesta are calculated similarly (i.e., as a function of $K_{OW,N}$ and lipid, NLOM, and water content).

Note that a proportionality constant relating the sorption capacity of lipid to octanol could also be defined but is omitted here as it is typically assumed to be unity. With respect to NLOM, empirical data indicate that the sorption capacity for neutral organic chemicals is approximately 20 to 40 times lower than that of lipids (i.e., $\rho_{NLOM} = 0.025–0.05$) [15,33]. In other words, NLOM becomes an important consideration only for organisms and tissues where the ratio of lipid to NLOM content approaches the ratio of the NLOM to lipid proportionality constant (i.e., lipid content is 20 to 40 times lower than the NLOM content). Note that as sorption to NLOM is estimated using the octanol–water partition coefficient (i.e., a solvent dissolution model), it best represents specific (e.g., hydrogen donor–acceptor) and nonspecific (e.g., van der Waals) interactions as opposed to site-specific binding to biological macromolecules (e.g., albumin, fatty acid binding proteins) where the nature and number of binding sites plus competition between endogenous and other exogenous ligands can be important considerations [34].

For IOCs, the sorption capacity of the organisms compared to water (D_{BW}, in L/kg) is based conceptually on the approach outlined in Schmitt [15] and therefore modified to: (1) explicitly consider phospholipids separately from neutral (storage) lipids, and (2) use distribution ratios to account for the presence of the neutral and charged form of the chemical. The modified equation is shown below

$$D_{BW} = f_{SL}D_{OW} + f_{PL}D_{MW} + f_{NLOM}\rho_{NLOM}D_{OW} + f_W \quad (7)$$

where f_{SL} and f_{PL} are the volume fractions of neutral (storage) lipids and phospholipids, respectively. Following Hendriks et al. [52], phospholipids were assumed to account for 1% of the organism on a whole-body basis whereas neutral lipids account for 4%, NLOM for 15%, and the remainder is water [16]. The octanol–water distribution ratio (D_{OW}) and membrane–water distribution ratio (D_{MW}) are estimated as a weighted average of the respective partition coefficients for the neutral and charged form, that is,

$$\begin{aligned} D_{OW} &= \chi_N K_{OW,N} + \chi_I K_{OW,I} \text{ and} \\ D_{MW} &= \chi_N K_{MW,N} + \chi_I K_{MW,I} \end{aligned} \quad (8)$$

where χ_N is the fraction of chemical in neutral form and χ_I is the fraction of chemical in charged form at biological pH (not external bulk water pH), as estimated using the Henderson–Hasselbalch equation. Methods for estimating the membrane–water partition coefficients of neutral organic chemicals are summarized in Supplemental Data, Section S5. These approaches are assumed to be valid for the neutral form of IOCs. The apparent octanol–water and membrane–water partition coefficients for the charged form are then estimated from the partition coefficients of the neutral form,

$$\begin{aligned} \log K_{OW,I} &= \log K_{OW,N} - \Delta_{OW} \text{ and} \\ \log K_{MW,I} &= \log K_{MW,N} - \Delta_{MW} \end{aligned} \quad (9)$$

where Δ_{OW} and Δ_{MW} are scaling factors relating the two partition coefficients [19,53]. The Δ_{OW} values reflect the increased solubility of the charged form in the aqueous phase but also reduced solubility in the octanol phase whereas the Δ_{MW} values can be viewed as representing the competing influence of increased solubility of the charged form in the aqueous phase versus the attractive electrostatic interactions with membrane phospholipids. The Δ_{OW} typically ranges from 2 to 4 log units (see above regarding the role of ion pairing), whereas Δ_{MW} typically ranges from $\leq$0.3 to 2 log units depending on the type of IOC [19,20] (see Supplemental Data, Section S2). Accordingly, whereas the neutral form can sorb to neutral (storage) lipids and phospholipids to a similar degree, the charged form is expected to sorb preferentially to phospholipids. This behavior is qualitatively consistent with the low wet-weight concentrations reported for PFAAs in adipose tissue of rainbow trout compared to liver and kidney [30,31]. Note that because Δ_{OW} is expected to be larger than Δ_{MW} for the majority of IOCs, mechanistic BCF modeling approaches using D_{OW} only are expected to result in bias toward underestimation, particularly for chemicals exhibiting a high degree of ionization and affinity for phospholipids.

Elimination

The elimination rate constant characterizing respiratory exchange back to the water column (k_W) was estimated in the original model [16] using the following equation

$$k_W = \frac{k_U}{K_{BW}} \quad (10)$$

For the mechanistic model for IOCs, k_W is estimated by using D_{BW} instead of K_{BW}. Similarly, the fecal egestion rate constant is calculated using distribution ratios rather than partition coefficients. Although the degree of ionization is still an important determinant of the steady-state BCF because it influences sorption capacity (D_{BW}), a notable feature of the approach outlined above is that chemical absorption efficiency (E_W) influences k_W as well as k_U. Thus slower uptake kinetics (i.e., reduced E_W) are counteracted by slower depuration kinetics and the steady-state BCF is determined by the sorption capacity of the organism (K_{BW} or D_{BW}), assuming that all other elimination rate constants are negligible in comparison to k_W. However, for chemicals that are biotransformed to a significant extent (e.g., $k_M \geq k_W$), the effect of ionization on reducing E_W is more important for determining the steady-state BCF because while the total uptake term (k_U) is reduced, the total elimination term k_E is not reduced proportionally.

As with uptake and elimination via respiratory exchange, the rate constants describing dietary uptake and fecal egestion in the original model [16] include a chemical absorption efficiency term. As this term is derived using the same basic approach as for E_W (i.e., a two-resistance model), a multiplier (μ) could also be applied to account for the degree of ionization. However, this simplified approach may not be as appropriate for describing chemical exchange in the GIT. For example, an analysis of empirical human GIT absorption efficiencies for acids and bases

[54] concluded that, as a first approximation, gut absorption efficiency can be estimated using the properties of the neutral form alone. In accordance with the empirical analysis of human GIT absorption efficiencies [54], uptake efficiencies calculated for stronger bases ($pK_a \geq 10$) using a theoretical passive absorption model of the human GIT [47] are not substantially reduced from what is calculated for neutral compounds with similar hydrophobicity. Relatively high residence times in the GIT (compared to at the gill interface), the large surface area of the small intestine, and the nature of its mucosal layer could partially explain these findings.

Biotransformation

Arnot and colleagues compiled a database [55] of whole-body biotransformation rate constants (k_M, 1/d) for organic chemicals in fish and subsequently developed a screening level quantitative structure–activity relationship (QSAR) model to predict k_M from chemical structure [56]. In that QSAR, K_{OW} is one of the parameters included in the prediction of k_M. For the present model calculations, QSAR estimates of k_M were generated using the default log $K_{OW,N}$ in EPISUITE 4.10 [9] for all compounds irrespective of degree of ionization.

MATERIALS AND METHODS

Empirical BCF database compilation

The main source of empirical BCF data is the Arnot and Gobas [57] database, which is comprised of data derived from publicly available sources and evaluated using several data quality criteria. A total of 450 observations ($n = 301$ for organic acids, $n = 149$ for organic bases) were compiled from this source representing 204 distinct compounds. The majority of data points are from studies using common carp, fathead minnow, zebrafish, and rainbow trout. Wet-weight empirical BCF data were adjusted to correspond to a standard 5% lipid-content basis if the whole-body lipid content was reported and differed from this value. Multiple data points from the same study and organism for the same chemical were averaged (geometric mean). After this step, a total of 150 data points for acids (130 distinct compounds) and 74 data points for organic bases (74 distinct compounds) remained. Other publicly available data compilations [9,12,58] were also searched to find additional BCF data for IOCs that were not already included and evaluated in Arnot and Gobas [57]. Extensive overlap exists between these sources, primarily due to the large number of shared data points obtained from the Japanese National Institute of Technology and Evaluation (NITE) database (http://www.safe.nite.go.jp/english). However, we did identify 55 additional compounds ($n = 29$ acids, $n = 26$ bases) compiled by Fu et al. [12]. Empirical BCFs for these compounds originate from the Pesticide Property Database (PPDB; http://sitem.herts.ac.uk/aeru/footprint/index2.htm). The PPDB includes pesticide data from regulatory agencies and is subject to a degree of data evaluation and verification. In the present study, we only included PPDB data that were classified as verified measured BCF values (i.e., reliability scores of 4 or 5) and assumed that these data are of comparable quality to those in the existing Arnot and Gobas [57] database. Seven compounds originally included in Fu et al. [12] were not used for model evaluation in the present study due to low quality scores (i.e., PPDB reliability scores of 1, 2, or 3), leaving BCF data for 26 organic acids and 22 organic bases. The empirical BCF data for PFAAs reported by Martin et al. [30] and Inoue et al. [31] were also compiled (see Supplemental Data, Section S6) but kept separate from the model test set described above.

Physical–chemical property database compilation

The following data were compiled for all chemicals in the BCF database: (1) $K_{OW,N}$, (2) biotransformation rate constant (k_M), (3) pK_a, (4) type of acid or base, (5) number of ionization centers, and (6) charge number at biologically-relevant pH (i.e., single or multiple charges). The $K_{OW,N}$ and k_M were taken from the EPISUITE 4.10 estimation and database software [9]; empirical values were given precedence over estimated values. Empirical data on k_M are lacking for the majority of compounds. Total pK_a values were generated using Advanced Chemistry Development (ACD) Labs V12 (Ver 12.5; Build 39480, April 1, 2010). Besides supporting batch mode calculations, the advantage of using ACD Labs software is that ionization centers are automatically classified as either acidic or basic in addition to pK_a values being estimated. We recorded the estimated apparent pK_a values for each ionization center as these values are expected to be most representative of IOCs in aqueous solution for compounds with multiple ionizable functional groups [59]. In general, estimated pK_a values are expected to be accurate within ± 1 log unit [59,60] although there may be some exceptions. Discrepancies between ACD Labs output and pK_a values reported in the Fu et al. [12] database were noted for some bases (e.g., metconazole, triforine, benzothiazole) as well as several compounds classified as acids (e.g., parathion, cyhalothrin, fenitrothion). Some of these pK_a values (e.g., metconazole $pK_a = 11.4$, triforine $pK_a = 10.6$, cyhalothrin $pK_a = 9.0$) originate from the PPDB. On further inspection, we note that it is not always indicated whether the pK_a refers to an acidic or basic functional group and that multiple pK_a values listed in the PPDB (e.g., two pK_as are listed for metconazole) cannot readily be associated with different ionizable functional groups on the molecule. Some IOCs in the PPDB are also misclassified (e.g., fenpropimorph and pyriproxyfen are identified as weak acids). The discrepancies and errors among different sources of pK_a estimates warrant further clarification but this task is outside the scope of the present study. In all cases where discrepancies were noted, ACD Labs output generated for this exercise was used. Acids were then categorized as being phenolics, carboxylic acids, sulfonic acids or other, whereas bases were categorized as primary, secondary, and tertiary amines or other. Multiprotic compounds that are effectively monoprotic at environmentally relevant pH (i.e., have additional acidic ionization centers with $pK_a \geq 10$ and/or basic ionization centers with $pK_a \leq 4$) were also identified in the database.

Estimated log $K_{OW,N}$ values for linear isomers of PFAAs from different models are presented in the Supplemental Data (Section S7). A notable discrepancy is between the two versions of EPISUITE KowWIN (v1.67 and v1.68), with estimated log $K_{OW,N}$ values in the most recent version (v1.68) being approximately 1.5 to 3 orders of magnitude lower (e.g., log $K_{OW,N} = 4.81$ vs 6.30 for PFOA; log $K_{OW,N} = 4.49$ vs 6.28 for PFOS). These differences are due to the inclusion of a correction factor for the chain length of the internal CF_2 core (-0.3 log units per CF_2). The justification for this correction factor is unclear and also leads to relative differences between linear and branched isomers of PFOA that are inconsistent with recently reported COSMOtherm calculations (see Supplemental Data, Section S7). Whereas output from KowWIN v1.68 is lower than the previous version, output from more recent applications of COSMOtherm (C2.1) is higher (1–1.5 orders of magnitude).

Estimated log $K_{OW,N}$ values generated by SPARC v4.6 (October 2011 release w4.6.1691–s4.6.1687) are well in excess of the KowWIN v1.67 values and were not considered further (e.g., SPARC v4.6 log $K_{OW,N} = 6.93$ for PFOA, 8.14 for perfluorononanoic acid). All estimated property values for these compounds are considered uncertain.

Selection of BCF model test set

Only monoprotic acids and bases plus those compounds that are effectively monoprotic at pH = 7.0 were included in the model test set (i.e., zwitterions and multiprotic acids and bases were eliminated). A quaternary (i.e., permanently charged) amine (difenzoquat) and those chemicals in the Fu et al. database [12] for which ACD Labs V12 did not identify any ionization centers were also excluded. Monoprotic organic acids with $pK_a > 11$ and monoprotic organic bases with $pK_a < 3$ were then screened out to prevent the BCF test set from being populated with IOCs that are less than 0.01% ionized at pH = 7. Following application of this last screening criterion, the final BCF model test set includes 149 observations for acids ($n = 129$ distinct chemicals, ~85% from Arnot and Gobas [57]) and 62 observations for bases ($n = 62$ distinct chemicals, ~75% from Arnot and Gobas [57]). The empirical BCF dataset for PFAAs was restricted to perfluorocarboxylic acids with seven or more perfluorinated carbons (i.e., PFOA and longer-chain homologs) and PFOS.

Model parameterization and application

Two separate model applications were conducted. The first application of the revised model was to calculate BCFs for all compounds in the model test set (see above). The second was to calculate BCFs for the PFAAs using the different sets of estimated log $K_{OW,N}$ (see Supplemental Data, Section S7). The application of the revised model for predicting BCFs for PFAAs is included primarily to explore the potential role of phospholipids in determining the bioaccumulation potential of these compounds as opposed to providing a definitive BCF model for these compounds. For example, specific protein interactions are not explicitly considered because only a generic approach for estimating protein–water partitioning is included in the model. Additionally, the proposed model does not consider the possibility of concentration-dependent bioaccumulation potential, as reported in a semi-static exposure study with green mussels [61]. Note that this concentration-dependent bioaccumulation behavior was not consistently observed in experimental BCFs reported for carp exposed in flow-through experiments [31]. Values for key biological (e.g., W_B, f_{SL} and f_{PL}) and physical–chemical (e.g., Δ_{OW} and Δ_{MW}) model input parameters are summarized in Supplemental Data, Section S8. The most important environmental parameter in the current simulations is the pH of the aqueous phase. For these calculations, the pH of the water in the test system was assumed to be 7.0 and the pH at the gill assumed to be one order of magnitude lower than in the exposure water. The BCFs calculated in the present study are based on the truly dissolved water concentration and therefore do not consider the potential for reduced bioavailability due to sorption to particulate or dissolved matter present in the test system.

To evaluate different models (hypotheses), both the original Arnot and Gobas [16] model and the revised version for IOCs were used to calculate BCFs for the model test set (see above). When applying the original BCF model (N), data characterizing the dissociation behavior of the test set compounds are ignored (i.e., the fraction of chemical in neutral form is assumed to be 100%) and hence model output is driven solely by hydrophobicity ($K_{OW,N}$) and susceptibility to biotransformation (k_M). The distinction between storage lipids and phospholipids is also disregarded. The purpose of these simulations is to assess the potential bias related to such model assumptions, which although obviously inappropriate for compounds exhibiting a high degree of ionization, greatly reduces the amount of effort required to characterize partitioning behavior and may still be useful for screening purposes (i.e., worst-case scenario). The default model calculations using the original model were also repeated assuming a negligible biotransformation rate constant (N, $k_M = 0$). Calculations with the revised model for IOCs were conducted under the following assumptions: (1) default parameterization (I); (2) default but with negligible biotransformation rate constant (I, $k_M = 0$); (3) default but assuming that the phospholipid content is zero (Ib); and (4) default but omitting the gill uptake correction factor (μ; Ic). Model assumptions for each scenario are summarized in the Supplemental Data. These simulations seek to gain insight into the potential importance of the different model assumptions when evaluated against sets of independent BCF data (i.e., no calibration or regression involved).

Only the revised BCF model was applied to the PFAAs included in this exercise. Simulations were conducted using different sets of log $K_{OW,N}$ estimates assuming the default model parameterization. Biological properties were adjusted to better match Martin et al. [30], which provided data on both fish characteristics and growth rates over the test period.

Evaluation of model performance

Following Arnot and Gobas [16], we have elected to use average model bias (MB) and average absolute model bias (AMB) to assess model performance for the model test set. Model bias is calculated as shown below

$$MB = \frac{\sum_{1}^{n} \log(BCF_M/BCF_E)}{n} \quad (11)$$

where BCF_M is the modeled bioconcentration factor, BCF_E is the empirical bioconcentration factor, and n is the number of observations. This metric represents the average factor by which model output deviates from the observations and is useful because it also indicates the directionality of any systematic bias. Model bias can be a misleading indicator of model accuracy because under- and overestimations cancel each other out. For this reason, MB is complemented by the AMB, calculated similarly except that the absolute deviation for each comparison of model output to empirical data is used, that is,

$$AMB = \frac{\sum_{1}^{n} ABS[\log(BCF_M/BCF_E)]}{n} \quad (12)$$

Root mean-square error (RMSE) and r^2 were also utilized to characterize model performance.

RESULTS AND DISCUSSION

Relationship between empirical BCF and hydrophobicity

Before discussing the performance of the original and revised BCF models, it is useful to illustrate the relationship between the empirical BCF data and hydrophobicity. The empirical BCF data compiled for the present study are plotted against log $K_{OW,N}$ and log D_{OW} (at pH = 7.0) in Figure 2. The

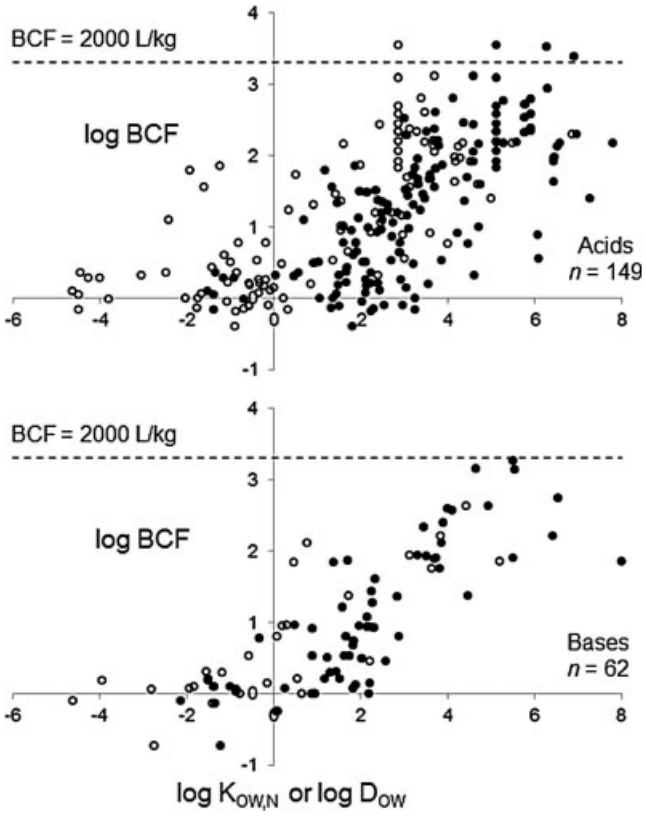

Fig. 2. Empirical bioconcentration factors (BCF, L/kg) for all acids and bases in the model test set ($n = 149$ observations for organic acids, $n = 62$ observations for organic bases) as a function of the octanol-water partition coefficient of the neutral form of the compound ($K_{OW,N}$; closed circles) and estimated octanol-water distribution ratio at pH = 7.0 (D_{OW}; open circles). B criterion of 2000 (dashed line) is also indicated.

BCF threshold determining whether or not a substance is categorized as bioaccumulative (B; BCF > 2,000 L/kg) under Annex XIII of the REACH regulation is also indicated. The uncertainty in the empirical BCF data is not represented in this figure, but is typically substantial [57]. Duplicate BCFs from the NITE database (same chemical, same organism) typically vary by a factor of $\leq$2 to 10, which corresponds to a factor of up to three lower and higher than the geometric mean used to summarize these data points. Multiple BCF data points for the same chemical but a different study or organism (which are represented as separate geometric means) vary over a similar range. This variability corresponds directly to the variability and uncertainty for these observations. The underlying variability and uncertainty in single-point BCF values (e.g., those in the PPDB) cannot be assessed.

As expected, empirical BCFs exhibit a positive relationship with both indicators of hydrophobicity [12,37]. Although deriving regression-based equations to describe the relationship between log BCF and hydrophobicity can be successful, it is also apparent that a substantial portion of the variability in empirical BCFs cannot be explained without introducing additional parameters, most importantly to represent susceptibility to biotransformation [55,56]. For example, empirical BCFs for acids with log $K_{OW,N} = 6$ span approximately three orders of magnitude. The majority of compounds included in the present study fall well below typical B categorization thresholds (Fig. 2), including those chemicals with relatively high log $K_{OW,N}$. Relatively low bioaccumulation potential compared to neutral organic compounds is not unexpected, given the effect of ionization on overall sorption capacity. Ionizable functional groups on the molecule such as hydroxyl groups can also result in inherently greater susceptibility to biotransformation [56]. With respect to ecological risk assessment, it is important to reiterate that relatively low bioaccumulation potential (i.e., categorization as "not B") may be countered by elevated inherent toxicity, particularly in the case of ionogenic pharmaceuticals and pesticides, which may be more likely to act through specific modes of toxicity (as opposed to via baseline narcosis). It is also important to note that dietary uptake could elevate the body burden substantially compared to that achieved considering uptake via respiratory exchange alone for the more hydrophobic compounds (i.e., bioaccumulation factor BAF > BCF) [16].

Evaluation and comparison of model performance: Model test set

Empirical BCF data are compared to model output generated using the default revised BCF model for IOCs in Figure 3. Average MB and average AMB for all model simulations (N = original model for neutral compounds, I = default revised model for IOCs, Ib = no phospholipids, Ic = no gill uptake correction factor; see Supplemental Data) are summarized in Figure 4 for different groups of test chemicals. Additional information on MB is summarized in the Supplemental Data (Supplemental Data, Section S9), including a figure illustrating the relationship between MB and hydrophobicity (Supplemental Data). Model performance assuming that the baseline E_W is an order of magnitude lower (i.e., $\beta = 0.0005$) is also summarized in the Supplemental Data.

As shown in Figures 3 and 4, the performance of the default revised model (I) for the model test set is acceptable given the inherent uncertainty and variability in the empirical BCFs (see above). Considering all acids and bases together, the default revised model (I) exhibits no systematic bias (i.e., MB = 0) and is accurate within a factor of three on average (AMB = 0.43) ($r^2 = 0.70$, RMSE = 0.63). Considering acids and bases separately, model output is still well correlated with observations ($r^2 = 0.68$ and 0.75 for acids and bases, respectively; Fig. 3), and average MB and AMB are also within a factor of three (Fig. 4). Model performance for stronger acids and bases ($pK_a \leq 6$ and $pK_a \geq 8$, respectively) deteriorates with respect to the RMSE and r^2 values (e.g., $r^2 = 0.62$ and 0.70, respectively; Fig. 3) but is comparable in terms of average MB and AMB (Fig. 4). The default revised model (I) systematically overestimates empirical data for stronger hydrophobic organic bases ($pK_a \geq 8$, log $K_{OW,N} \geq 3$) by a factor of five on average. However, there are only four compounds in this category (e.g., 1-octanamine, N,N-dioctyl-, CAS 1116-76-3). Model performance for all organic bases with log $K_{OW,N} \geq 3$ ($n = 18$) is more similar to overall model performance (i.e., MB within a factor of two, AMB within a factor of three; see Supplemental Data). Although model performance for the stronger hydrophobic organic acids ($pK_a \leq 6$, log $K_{OW,N} \geq 3$) is good on average, we note that modeled BCFs for carboxylic acids with log $K_{OW,N} \geq 6$ (e.g., 1-phenanthrenecarboxylic acid, CAS 1945-53-5) are systematically biased toward overestimation (Fig. 3; Supplemental Data).

Plausible explanations exist for some discrepancies between modeled and measured BCFs. The greatest potential for model errors are expected to be uncertainties in the estimated biotransformation rate constant (e.g., k_M too high or too low) or sorption capacity of the organism (e.g., due to log $K_{OW,N}$ or log $K_{MW,I}$ too high, Δ_{MW} not representative). Error in pK_a values can also contribute to model bias. For example, underestimating

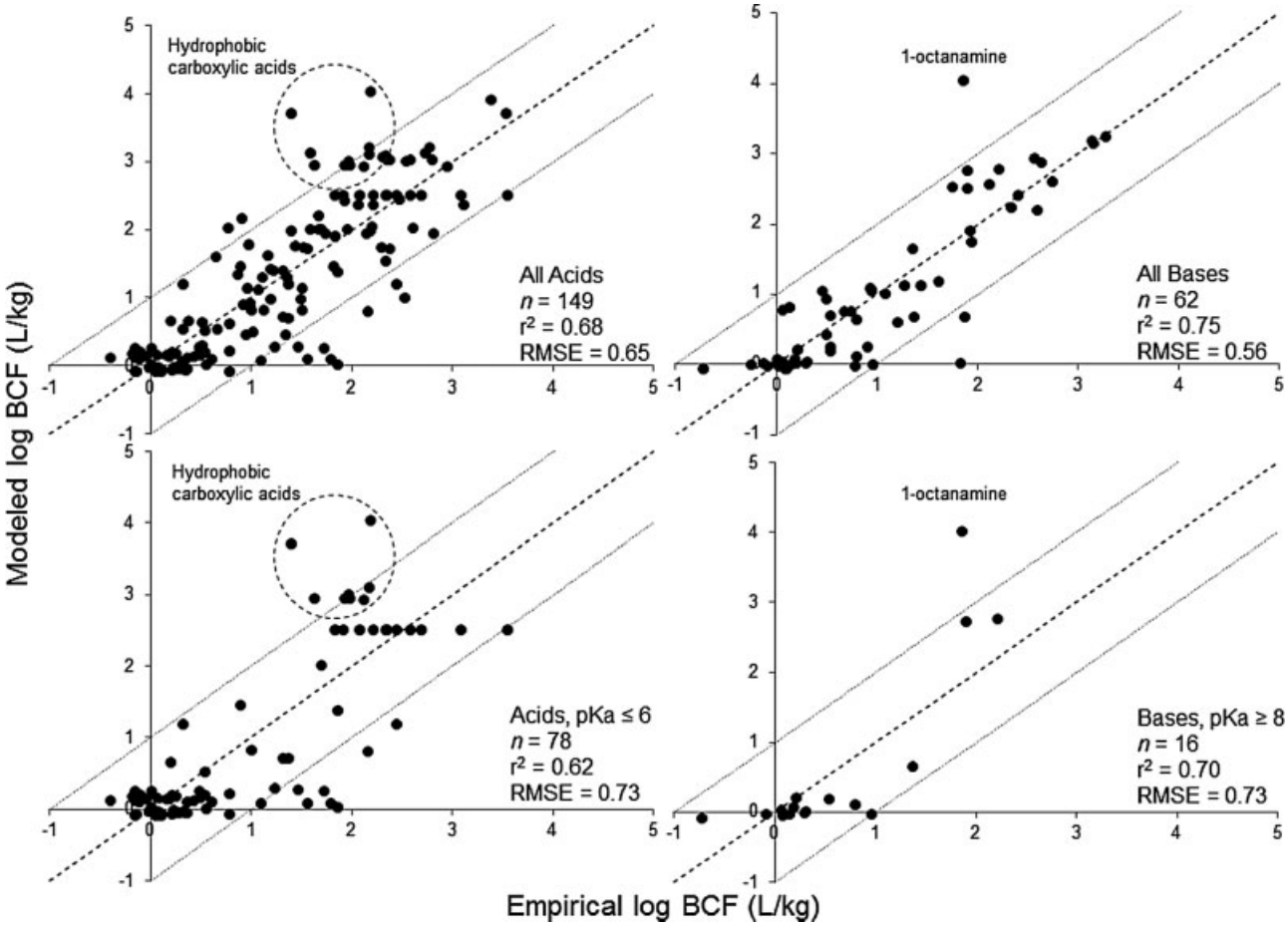

Fig. 3. Modeled vs. measured bioconcentration factors (BCF, L/kg) for organic acids and bases in the model test set ($n = 149$ observations for organic acids, $n = 62$ observations for organic bases). Model values were generated using the default revised model (I) parameterization. The 1:1 line (dashed) and factor of 10 lines (dotted) are also indicated. 1-octanamine refers to 1-octanamine, N,N-dioctyl-, CAS 1116-76-3 (a tertiary amine). RMSE = root mean-square error.

the actual pK_a of an organic acid leads to an underestimation of the fraction of the compound in neutral form and hence lower overall sorption capacity. In contrast, underestimating the actual pK_a of an organic base leads to an overestimation of the fraction of compound in neutral form and hence higher overall sorption capacity. Measurement error cannot be excluded for the more hydrophobic chemicals [57,62] such as 1-octanamine, N,N-dioctyl- (estimated log $K_{OW,N} > 7.0$) due to technical difficulties in obtaining reliable BCF measurements. As noted previously, the model calculations are based on the truly dissolved water concentrations whereas empirical BCF data are calculated from total water concentrations. Empirical BCFs based on total water concentrations can be lower than dissolved water concentration BCFs for chemicals sorbed to particulate and dissolved matter and hence unavailable for uptake into the organism. The relative importance of these sources of potential error (i.e., model vs measurement) cannot be ascertained from the currently available data (e.g., levels of particulate and dissolved organic matter not reported). With respect to hydrophobic carboxylic acids (estimated log $K_{OW,N} \geq 6.0$), no empirical k_M data are available for these compounds so estimated values were used in all cases. Carboxylic acids are not well represented in the training and validation sets for the k_M-QSAR [55,56]; therefore, these k_M predictions may have substantial errors.

Differences in average MB and AMB between the original and revised model parameterizations (N vs I; N, $k_M = 0$ vs I, $k_M = 0$; see Fig. 4) for acids and bases can be generalized as follows. Using the properties of the neutral compound only (i.e., ignoring ionization; N) shifts the MB toward overestimation compared to the default revised BCF model (I), most notably for the hydrophobic organic acids and bases, which exhibit a high degree of ionization (acids with p$K_a \leq 6$ and bases with p$K_a \geq 8$, respectively). This pattern in the model performance makes intuitive sense, given that the assumed sorption capacity of the charged form is up to three orders of magnitude lower (see default values for Δ_{OW} and Δ_{MW} in the Supplemental Data). Not surprisingly, assuming negligible biotransformation rate constants (N, $k_M = 0$) results in larger overestimation (e.g., MB and AMB ≥ 1.5 log units). In the context of ecological risk assessment, ignoring ionogenicity and biotransformation can therefore be expected to yield conservative estimates of exposure potential for such compounds, particularly if combined with the assumption that the compound is 100% bioavailable in the water column (as opposed to being strongly sorbed to particulate and dissolved matter). These simplifying assumptions could be applied as part of a tiered assessment approach; IOCs that exceed exposure thresholds under these simplifying assumptions could be reassessed using more realistic model parameterizations. It is also noteworthy that the original BCF model (N) performs much better when one considers all bases in the model test set (MB and AMB ≤ 0.5 log units) compared to other compound categories. This pattern in model performance is related to the fact that many of these compounds are weak bases in which the neutral form dominates or bases with such low hydrophobicity that the water content contributes

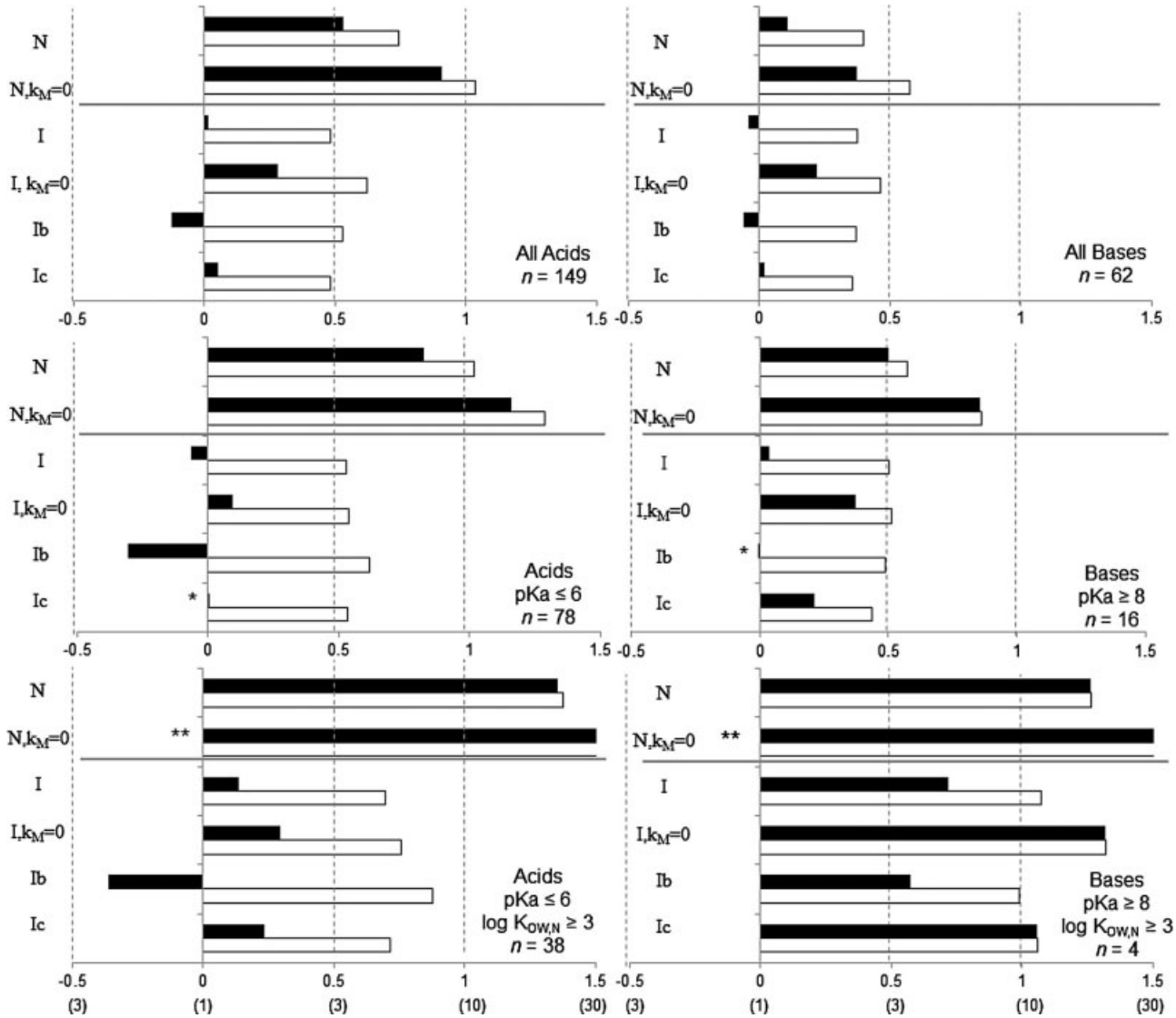

Fig. 4. Average model bias (MB) (solid bars) and average absolute model bias (AMB) (open bars) of the modeled BCFs under different model assumptions and parameterizations, plotted in log units. Negative average MBs indicate systematic underestimation; positive average MBs indicate systematic overestimation. Asterisk (*) indicates that MB is close to 0 (no systematic under- or overestimation). Double asterisk (**) indicates that MB and/or AMB is >1.5 log units. The corresponding factor of agreement is indicated in parentheses. N refers to the model parameterization using properties of the neutral compound only; N, $k_M = 0$ is the same scenario as N except biotransformation rate constants for all test chemicals are assumed to be negligible. I refers to the default revised model for IOCs; I, $k_M = 0$ is the same scenario as I except biotransformation rate constants for all test chemicals are assumed to be negligible. Ib refers to the model parameterization excluding phospholipids. Ic refers to the model parameterization excluding the simplified correction for gill uptake and elimination. See Supplemental Data for details of each model scenario.

substantially to the overall sorption capacity (i.e., description of partitioning to lipids and NLOM less relevant). For the latter category of compounds, biotransformation is also contributing relatively less to total elimination than other processes, notably gill elimination. As summarized in the Supplemental Data (Section S9), model performance for basic compounds with log $K_{OW,N} \leq 3$ is similar for all model calculations (MB within a factor of 1.5 or less, AMB within a factor of 2). In general, the BCF for lower $K_{OW,N}$ (or D_{OW}) chemicals is predominantly determined by K_{BW} (or D_{BW}) and the uptake rate constant (k_U), and biotransformation (k_M) is insignificant compared to gill elimination (k_W).

With respect to model calculations using the revised BCF model (I) but different assumptions about phospholipid content (Ib) and gill uptake efficiency (Ic; Fig. 4), model performance can be summarized as follows. Omitting phospholipids from the model parameterization (i.e., only storage lipids and D_{OW} considered) shifts average MB toward underestimation, most notably for compounds exhibiting a high degree of ionization. This trend is most evident for the stronger acids included in this evaluation for which the neutral form has at least moderate hydrophobicity (i.e., p$K_a \leq 6$, log $K_{OW,N} \geq 3$). This pattern in model performance can be related to the key role of phospholipids in determining overall sorption capacity of the organism for such chemicals. As the majority of the stronger organic bases have pK_a values between 8 and 10, the neutral form of these compounds and hence the storage lipid fraction (f_{SL}) tends to contribute more substantially to the overall sorption capacity. Model output with and without phospholipids is within a factor of 1.5 or less, explaining the similar model performance. In contrast, the model output for organic acids with and without phospholipids exhibits greater differences (up

to a factor of 15 depending on pK_a, type of acid, log $K_{OW,N}$, and k_M), with the model evaluation indicating that inclusion of phospholipids results in improved model performance. Omitting the gill correction factor (μ) from the model parameterization (Ic) shifts average MB toward overestimation, again most notably for the stronger acids and bases for which the neutral form has at least moderate hydrophobicity (i.e., log $K_{OW,N} \geq 3$). This pattern in model performance can be explained by the influence exerted by μ on E_W and hence the uptake rate constant (k_U) for such compounds (Fig. 1). Omitting the gill correction factor results in elevated E_W (up to a factor of ~100 for some IOCs), which acts to not only increase the uptake rate constant k_U but also the relative importance of gill elimination (k_W) with respect to the total elimination rate constant (k_E). For chemicals that are eliminated predominantly via biotransformation (high k_M) however, the overall effect of increasing E_W is a higher modeled BCF because the relative change in numerator (k_U) is greater than the relative change in the denominator (k_E). Because of the uncertainties in key model inputs (i.e., k_M, $K_{OW,N}$, Δ_{MW}) and the limited number of observations, it is difficult to comment further on the reliability of the simplified gill uptake model (or the potential importance of diffusive uptake of the charged form across the gills) for IOCs exhibiting a high degree of ionization (i.e., $\mu > 10{,}000$). Additional data and analyses are required.

Evaluation of model performance: Perfluoroalkyl acids

Modeled BCFs for the linear perfluorinated carboxylic acid homologs included in this exercise are compared to empirical BCF (carcass) data [30] in Figure 5. Carcass BCF data were selected for the model evaluation as they are the most appropriate surrogate for whole-body BCF (e.g., reportedly contain 85–96% of total mass of chemical). Modeled BCFs were generated assuming a pK_a value of 1.0 for all compounds; model predictions for PFOS ($pK_a = 0$) and for perfluorinated carboxylic acids under different model assumptions (e.g., $pK_a = 3.8$, phospholipid content = 0) are summarized in the Supplemental Data, Section S10 and S11. Modeled BCFs as a function of log $K_{OW,N}$ are also presented (see Supplemental Data, Section S11). The model output for log $K_{OW,N} \leq 4$ (see Supplemental Data) can be viewed as generic estimates for shorter chain-length PFAA homologs not included in Figure 5.

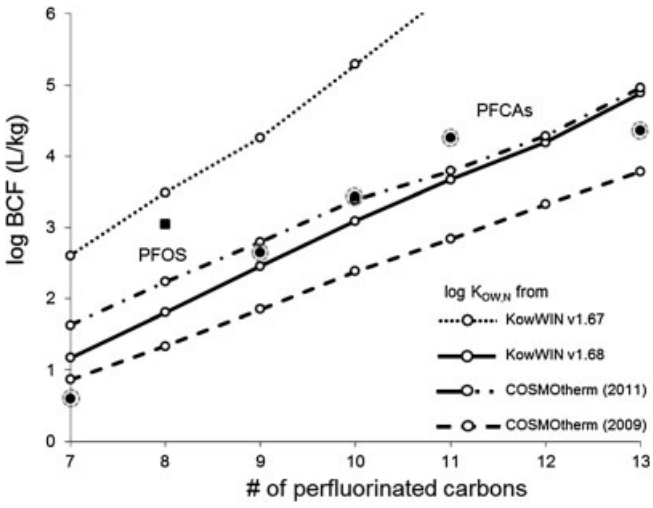

Fig. 5. Comparison of modeled bioconcentration factors (BCF, L/kg) and empirical BCF data for perfluorinated acids in the carcass of the fish [30] with increasing number of perfluorinated carbons. Model output for PFCAs (open circles) assuming $pK_a = 1.0$ for all homologues but using different values for log $K_{OW,N}$ are indicated by the open circles connected using different lines. Empirical data for PFCAs are represented by the closed circles surrounded by a dashed circle. Note that empirical data for PFOS is shown (closed square) but model output for this compound is omitted from this plot (see Supplemental Data, Section S10).

Due to the high degree of ionization exhibited by these substances when assuming $pK_a \leq 1$, the estimated E_W of some of the PFAAs falls to the assumed baseline value ($\beta = 0.005$). However, because both growth dilution and biotransformation are assumed to be negligible and because E_W is used to estimate both uptake and elimination across the gills, potential error in E_W cancels out and the steady-state BCF is relatively insensitive to this value. Model output is then driven primarily by the distribution ratios (D_{MW}, D_{OW}) and lipid/NLOM volume fractions (e.g., f_{NL}, f_{PL}). As can be seen in Figure 5, the agreement between model output and the empirical BCF data depends on which set of estimated log $K_{OW,N}$ values are used. For example, model output generated using the log $K_{OW,N}$ values from KowWIN 1.67 is systematically biased high by nearly two orders of magnitude. Modeled BCFs using the reported 2006/2009 COSMOtherm-based estimates (see Supplemental Data, Section S7) are in good agreement for PFOA but then are systematically biased low for the other homologs. In contrast, modeled BCFs using the reported 2011 COSMOtherm estimates (see Supplemental Data, Section S7) are biased high for PFOA but are more representative of the empirical data for the higher homologues. Good overall agreement between modeled and empirical BCFs is also obtained when using the KowWIN v1.68 estimates [9]. Gill ventilation (k_W) is the dominant elimination pathway, but fecal egestion becomes increasingly important for longer chain-length homologs (i.e., as hydrophobicity increases, influence of fecal egestion increases). Model output for PFOS is biased low by a factor of approximately 2 to 150, depending on choice of log $K_{OW,N}$. The best model performance for PFOS is obtained when using the 2011 COSMOtherm property value (log $K_{OW,N} = 6.43$) (see Supplemental Data, Section S7). However, as noted above, this set of property values leads to substantial overestimation of the BCF of PFOA. The observation that PFOS has a BCF two orders of magnitude higher than PFOA yet similar (or lower) estimated hydrophobicity (log $K_{OW,N}$; see Supplemental Data, Section S7) cannot be reconciled by the proposed model if such a relationship between property values is adopted. Model output generated assuming no phospholipids (i.e., $f_{PL} = 0$) is approximately 2.5 to 4 times lower than the default revised model output (see Supplemental Data, Section S12). Given the generic assumptions regarding the distribution ratios, approximately 70 to 75% of the steady-state body burden is associated with phospholipids when included compared to 5% for NLOM (i.e., proteins, carbohydrates). Broadly speaking, these model results highlight the potential importance of phospholipids in determining the bioaccumulation potential of these compounds. Further empirical investigations are obviously required. Depending on the outcome of such studies, normalizing empirical wet-weight PFAA concentrations to tissue phospholipid content could be a justifiable approach when assessing bioaccumulation potential (i.e., analogous to lipid normalization for neutral organic chemicals). Opportunities to explore this hypothesis further using the currently available biological monitoring data appear to be limited given that phospholipid contents are not reported.

The overall patterns in model performance for perfluorinated carboxylic acids can be explained in part by comparing the

change in log $K_{OW,N}$ per additional CF_2 to the change in empirical BCF per additional CF_2. For example, each additional CF_2 increases the estimated $K_{OW,N}$ by a factor of approximately 3 to 4 for the COSMOtherm-based values and by a factor of 5 for KowWIN v1.68, whereas the empirical BCF*s* increase by a factor of approximately 7 to 10 (for number of $CF_{2,3} = 7$ to 11). Bias in model output could therefore be attributed solely to the bias in log $K_{OW,N}$ estimation. However, another possibility is that the generic value for the NLOM proportionality constant ($p_{NLOM} = 0.05$) [33] is not representative for some or all of these compounds (e.g., leads to underestimation of protein-water distribution coefficients). Inclusion of approaches to characterize protein-specific interactions in fish under physiologically relevant conditions might improve model performance, but, as discussed previously, would be appropriate only if all relevant considerations (e.g., competition for binding sites with endogenous ligands) are accounted for. Selecting appropriate surrogates to characterize these interactions is also important as bovine serum albumin and rat liver fatty acid binding proteins may not be representative for fish (particularly on a whole-body basis) [35]. Empirical studies characterizing the sorption of PFAAs to structural proteins (e.g., collagen, actin, myosin) [63] could be useful, as this general class of proteins dominates in terms of volume fraction (whole-body basis). Note that sorption to these proteins or any other additional phases of interest can be included in the proposed model using the volume fraction and the estimated distribution ratios.

Research priorities

The proposed mechanistic BCF model for IOCs is conceptualized using established principles from the pharmaceutical and bioaccumulation literature; however, the current results rely heavily on generic estimation methods for several key input parameters. Model performance would benefit most from improved estimates of k_M (i.e., better characterization of susceptibility to biotransformation) and chemical-specific values for Δ_{MW} (i.e., relating $K_{MW,I}$ to $K_{MW,N}$). Additional uncertainties stem from the reliance on estimated pK_a values and the variability in log $K_{OW,N}$ estimates generated by different property estimation software. The model is particularly sensitive to values of k_M for the more hydrophobic chemicals, which, in the absence of biotransformation, tend to have greater inherent bioaccumulation potential in fish. Targeted studies to expand the database of empirical k_M values to include more IOCs would be useful (e.g., for a set of primary to tertiary amines with various substitution patterns). Revisions to the existing k_M-QSARs or derivation of new estimation algorithms specifically for IOCs could then be implemented. Priorities for obtaining chemical-specific values of Δ_{MW} are for carboxylic and sulfonic acids, given the relative lack of data [53], and for "bulkier" strong organic bases (e.g., tertiary amines) where $K_{MW,I}$ is more likely to be $<< K_{MW,N}$. Automated computational approaches to estimate these parameters from chemical structure would also be highly valuable. With respect to the estimation of $K_{MW,N}$, polyparameter approaches (i.e., polyparameter linear free energy relationships), which estimate $K_{MW,N}$ independent of $K_{OW,N}$ [22], could be applied in future model calculations (assuming that these methods are suitable for the neutral form of IOCs and the required solvation parameters can be reliably estimated). Empirical data on chemical absorption efficiency (E_W) and gill uptake rate constants (k_U) are lacking in general and for IOCs in particular (especially cationic IOCs). To further test and refine the simplified gill uptake model, it is therefore recommended that additional experiments be conducted for different IOC chemical classes (e.g., amines and other cationic IOCs, organic acids with $pK_a < 3$) following the approach of Erickson and colleagues [38,39]. The results of high-quality empirical studies with quaternary amines (or other hydrophobic ions that can be assumed to be permanently charged) could also prove useful for improving estimates of uptake efficiency (E_W) for IOCs exhibiting a very high degree of ionization at environmentally relevant pH. Charge density and other three-dimensional molecular factors may also play a role in diffusive uptake and elimination processes and should be probed experimentally. Experiments including concurrent exposure to neutral reference compounds could facilitate data interpretation and are therefore encouraged.

Despite the uncertainties in the model, it has to be recognized that only mechanistically based modeling approaches are flexible enough to be applied for both screening and categorization exercises (e.g., "B" or "not B") and ecological risk assessment. For example, regression-based approaches based on laboratory BCF data cannot readily be applied to different fish or aquatic environments differing in physical characteristics. Furthermore, the potential influence of dietary uptake and hence food web bioaccumulation patterns cannot be assessed (e.g., BCF-based regressions cannot be applied to estimate bioaccumulation factors and trophic magnification factors in different food chains). The model evaluation exercise indicates that mechanistic bioaccumulation modeling approaches developed for neutral chemicals can successfully be adapted to IOCs and hence developed further. Application of the proposed model to characterize the bioaccumulation behavior of pharmaceuticals of concern in aquatic environments [7,8] is a priority. In addition, multicompartment (physiologically based pharmacokinetic) models including different lipid and protein classes (e.g., plasma proteins vs tissue proteins) could be applied to better characterize the uptake and tissue distribution of IOCs in wildlife. However, to realistically simulate field conditions for exposure and risk assessment, there is a need to develop improved estimates of dissolved chemical concentrations from total water concentrations for IOCs, particularly for those that can engage in attractive electrostatic interactions with suspended particles [64]. Ionic strength is an important consideration (e.g., for applications to marine systems) in this regard. Failure to take account of these processes may result in substantial bias in modeled tissue residues of IOCs.

SUPPLEMENTAL DATA

Additional details on model development, parameterization, application, and evaluation are provided in the Supplemental Data. The compilation of empirical BCF values is also provided in electronic format (705 KB PDF).

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