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ASSESSING THE BIOACCUMULATION POTENTIAL OF IONIZABLE ORGANIC COMPOUNDS: CURRENT KNOWLEDGE AND RESEARCH PRIORITIES

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Abstract: The objective of the present study was to review the current knowledge regarding the bioaccumulation potential of ionizable organic compounds (IOCs), with a focus on the availability of empirical data for fish. Aspects of the bioaccumulation potential of IOCs in fish that can be characterized relatively well include the pH dependence of gill uptake and elimination, uptake in the gut, and sorption to phospholipids (membrane–water partitioning). Key challenges include the lack of empirical data for biotransformation and binding in plasma. Fish possess a diverse array of proteins that may transport IOCs across cell membranes. Except in a few cases, however, the significance of this transport for uptake and accumulation of environmental contaminants is unknown. Two case studies are presented. The first describes modeled effects of pH and biotransformation on the bioconcentration of organic acids and bases, while the second employs an updated model to investigate factors responsible for accumulation of perfluorinated alkyl acids. The perfluorinated alkyl acid case study is notable insofar as it illustrates the likely importance of membrane transporters in the kidney and highlights the potential value of read-across approaches. Recognizing the current need to perform bioaccumulation hazard assessments and ecological and exposure risk assessment for IOCs, the authors provide a tiered strategy that progresses (as needed) from conservative assumptions (models and associated data) to more sophisticated models requiring chemical-specific information. *Environ Toxicol Chem* 2017;36:882–897. © 2016 SETAC

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INTRODUCTION

The behavior of ionizable organic chemicals (IOCs) in humans has long been of interest to the pharmaceutical industry, and in silico tools to simulate the uptake, distribution, and elimination of such substances are widely available [1–3]. This is because many active pharmaceutical ingredients (APIs) are ionized at physiological pH values [4,5]; thus, understanding the impact of speciation (i.e., the fractional amount of neutral and ionized forms) on pharmacokinetics is important. Increasingly, environmental chemists, toxicologists, and ecological risk assessors have begun to focus their attention on IOCs. The main drivers of this interest include the detection of perfluorinated alkyl acids (PFAAs) in humans and wildlife around the globe [6,7], concerns regarding the potential impacts of APIs in aquatic environments [8,9], and the recognition that IOCs make up a substantial portion of the chemicals in commerce requiring assessment under various regulatory schemes (e.g., the European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals [REACH] regulation) [10]. Because it is not feasible to directly test all chemicals of interest, there is a need to develop and build confidence in models that can be used to support hazard and risk assessment of IOCs. Whereas numerous modeling tools are available to support the assessment of neutral organic chemicals, generic tools designed for IOCs have only recently been developed [11–14] and are not yet in widespread use.

The main objective of the present study was to review current knowledge regarding the bioaccumulation potential of IOCs, with a focus on the availability of empirical data for fish and identification of issues potentially important for future model development. This effort can be traced to a workshop on IOCs held 5 to 7 November 2014 in Vancouver, British Columbia, Canada (Experts Workshop on the Ecotoxicological Risk Assessment of Ionizable Organic Chemicals: Towards a Science-Based Framework for Chemical Assessment). Since that workshop was held, additional work has been performed to evaluate existing models for accumulation of IOCs in fish, providing further insights into the key factors influencing uptake and elimination processes. Special emphasis is placed on chemical behaviors that distinguish IOCs from neutral substances, including preferential sorption to membranes (phospholipids) and plasma proteins, the influence of pH on uptake and elimination rates, and the greater potential importance of active transport mechanisms. Biotransformation pathways and possible differences in the type and abundance of enzymes responsible for these reactions in fish and mammals are

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also discussed. Because of a lack of empirical data, the present review does not address the potential accumulation of IOCs by aquatic invertebrates. However, many of the key considerations for fish will also be relevant for these organisms. Factors determining the uptake and accumulation of IOCs in terrestrial plants have been investigated and reviewed elsewhere [15–18]. A brief summary of this information is provided in the Supplemental Data (Section S1).

GENERAL CONSIDERATIONS

Bioaccumulation is an integral aspect of hazard assessment (e.g., persistence, bioaccumulation, and toxicity categorization) and exposure/risk assessment [19]. An understanding of the bioaccumulation of IOCs is also useful for interpreting toxicity data (e.g., relating external concentrations to internal body burdens). Bioaccumulation potential is commonly characterized using metrics such as the whole-body bioconcentration factor (BCF), the bioaccumulation factor (BAF), the biomagnification factor, and the trophic magnification factor (see Supplemental Data, Section S2, for definitions) [19,20]. For regulatory purposes, priority is typically given to BCFs and BAFs, and numerical thresholds range from 500 L/kg (CLP/Globally Harmonized System of Classification and Labelling of Chemicals) to 5000 L/kg (e.g., REACH vB criterion, Stockholm Convention B criterion) [21]. For exposure/risk assessment, chemical concentrations in blood or plasma have also been proposed as a key metric for IOCs [22,23].

As with neutral organic compounds, the bioaccumulation potential of IOCs is generally expected to be a function of hydrophobicity and susceptibility to biotransformation [24]. For example, despite substantial scatter in the empirical BCF data for IOCs [11,25], there are significant positive relationships between measured BCFs, octanol–water partition coefficients for the neutral form of the compound ($K_{OW,N}$), and octanol–water distribution ratios (D_{OW}; i.e., the pH-dependent weighted averages of the partition coefficients of the neutral and charged forms). Ionizable organic compounds also exhibit an inverse relationship between bioavailability, defined as the freely dissolved concentration in the water column, and hydrophobicity (D_{OW}) [26–28]. Thus, our knowledge of the behavior of neutral organic chemicals already provides a basic conceptual understanding of the general behavior of IOCs, and the main challenge is to modify available models to account for any important deviations that may exist.

Both regression-based [25,29,30] and mechanistic [11,31–33] models for predicting the bioconcentration of IOCs in aquatic organisms are available. Regression-based BCF models are derived through a statistical analysis of available data ("training set"), typically using physical–chemical properties (e.g., $K_{OW,N}$ or D_{OW} and dissociation constant [pK_a]) as the independent variables (predictors). Mechanistic BCF models are those that directly estimate and/or include estimated rate constants describing key uptake and elimination processes (e.g., exchange across the gills, biotransformation, fecal egestion, growth dilution). These models typically require physical–chemical property data, biological characteristics (e.g., body size, lipid content), and environmental conditions (e.g., temperature, alkalinity/buffering capacity, dissolved oxygen content) as inputs. The organism may be represented as a single well-mixed compartment or multiple compartments representing different tissues and organs. Model performance is generally evaluated by comparing predicted BCFs to empirical data. For both basic model types, predicted BCF values are often within a factor of 3 of observations on average, although substantially larger discrepancies can occur for some chemicals [11,25].

The main advantages of mechanistic models include greater flexibility and applicability (e.g., to different species and experimental/environmental conditions) and the ability to more readily identify critical processes and important data gaps. While regression-based BCF models can be useful for regulatory purposes and exposure/risk assessment, the lack of process information (i.e., outputs describing the underlying toxicokinetics) limits their utility for addressing the objectives of the present review. For this reason, they are not examined in any further detail. Regardless of the model type, a key concern for bioaccumulation assessment is the likelihood of type I ("false positive"/overestimation of true BCF) and type II ("false negative"/underestimation of true BCF) errors, with type II errors given priority in the regulatory domain. Although the performance of available BCF models for IOCs is encouraging, factor of 3 errors can have important regulatory implications (e.g., vB, B, or not B classifications under REACH; subject to or not subject to the provisions of the CLP/Globally Harmonized System of Classification and Labelling of Chemicals) [21]. The larger discrepancies observed for certain chemicals are more problematic in this context as well as with respect to exposure/risk assessment. Accordingly, research supporting the development of improved BCF models and parameterization tools (e.g., for estimating biotransformation rate constants) is warranted.

The following sections describe various toxicokinetic processes underlying the bioaccumulation of IOCs. For consistency with the broader literature, the discussion topics are divided into sections that address absorption, distribution, metabolism (biotransformation), and excretion (ADME). Following the general ADME discussion, 2 case studies are presented to explore key issues related to the bioaccumulation of IOCs in aquatic organisms. Because of the importance of gill exchange for the bioaccumulation of IOCs in aquatic organisms, the first case study focuses on the influence of bulk water pH on gill uptake, gill elimination, and bioconcentration of IOCs. The influence of biotransformation is also considered. The second case study focuses specifically on PFAAs and the role of renal clearance. The mechanistic models applied in these case studies [31,33] are summarized briefly in the Supplemental Data (Section S3). Two considerations thus far little explored in aquatic organisms but potentially relevant for IOCs are the presence of mucus at epithelial exchange surfaces (gills, gut, and skin) and the role of membrane transporters. These considerations are introduced first because both may influence several ADME processes.

Presence of mucus on exterior surfaces of aquatic organisms and epithelium

The presence and possible functions of mucus on the epithelial surfaces of aquatic organisms has received substantial attention in the ecological literature [34–36]. Mucus is composed of high–molecular weight macromolecules and water and forms a gel-like coating estimated to be approximately 10 μm to 500 μm thick. The predominant macromolecules in the mucus of vertebrates are glycoproteins (mucins), whereas mucopolysaccharides are more prevalent in the mucus of invertebrates. Because glycoproteins contain acidic functional groups (e.g., sialic acid), the mucus coating can act as a cation exchanger [34,35] and therefore engage in attractive interactions with organic bases and repulsive interactions with organic acids. We are not aware of any systematic studies

exploring how mucus influences chemical sorption and uptake/elimination at epithelial surfaces, including the skin, gills, and gastrointestinal tract. Given that the passive flux of many IOCs across biological membranes is dominated by diffusion of the neutral form [31,32,37,38], it is possible that the presence of mucus is relevant only for chemicals that are strongly dissociated at environmental and physiological pH values.

Potential role of membrane transporters/active transport

Biological membranes act as diffusion barriers for inorganic ions and polar and/or charged organic and inorganic molecules, including energy substrates and structural components (e.g., sugars, fatty acids, and amino acids), signaling molecules (e.g., hormones and neurotransmitters), and substances that regulate cell motility, osmotic pressure, and pH (e.g., various organic and inorganic ions and water). As such, these membranes provide for compartmentalization and control of biological function at several levels of biological organization. The transport of ions and polar molecules across biological membranes is largely controlled by membrane-bound channel and transporter proteins. These proteins may facilitate the movement of an ion or molecule down its electrochemical gradient or transport a substance against its electrochemical gradient via some type of active mechanism. The energy required for this active transport may be provided by hydrolysis of adenosine 5′-triphosphate (ATP; primary active mechanism) or by coupling the electrochemical potential of a solute to the transmembrane flux of a second solute (secondary active mechanism) [39–42].

Membrane transporters play a critical role in determining the pharmacokinetics of some APIs in humans [40,41,43]. This is true in part because many drugs are structurally similar to endogenous substances (indeed, this is frequently the basis for their activity). Based on a large and growing literature, it is clear that the major transporter families responsible for transmembrane flux of both drugs and toxicologically relevant compounds are as follows: the ATP-binding cassette transporter family [39,44] and the solute carrier (SLC) family [45] (capital letters are used to denote human transporter proteins, whereas fish transporter protein names are written as capitals followed by lowercase letters), including organic anion transporter polypeptides (OATPs/Oatps [SLCO family]), organic anion transporters (OATs/Oats [SLC22A superfamily]), and organic cation transporters (OCTs/Octs [SLC22A superfamily]).

The ATP-binding cassette transporters, including the well-known multixenobiotic resistance–related or multidrug resistance–related transporters, act via the primary active mechanism and generally operate as "efflux pumps" to keep intracellular concentrations of substrates low. Solute carrier proteins participate in both uptake and efflux of chemical substrates, although most are considered to be uptake transporters. The SLC transporter–mediated entry of ions into cells is a secondary active process linked to the transmembrane electrochemical potential of other ions [46].

A considerable number of studies on genetic sequences, tissue expression patterns, and toxicologically and physiologically relevant roles of ATP-binding cassette transporters in teleosts have been published in recent years, with much of the research focusing on multixenobiotic resistance–related transporters [47]. These studies have demonstrated functional similarities between mammals and teleosts for some multixenobiotic resistance–related orthologs, although questions remain concerning the occurrence of multiple isoforms of orthologs that exist in mammals only as 1 isoform [47,48]. The expression of multixenobiotic resistance–related transporters has been demonstrated in teleost embryos, suggesting that they play a role in protecting developing embryos against contaminants dissolved in water [49,50]. Additional research with isolated renal proximal tubules has shown that ATP-binding cassette transporters secrete xenobiotics in the teleost kidney [51–55].

In contrast to ATP-binding cassette transporters, there have been relatively few studies of SLCs in fish. Teleostean Oatps have been phylogenetically characterized [56–58], and functional studies performed using in vitro expression systems suggest a potential role in transporting pollutants and other environmentally relevant compounds [56,59–62]. Whole-animal studies conducted using classical substrates for mammalian transporters also indicate the occurrence and activity of Oats and Oatps in fish liver and kidney. Compared with the data available for mammals, knowledge of membrane transporters and their potential role in determining the bioaccumulation potential of IOCs in fish is limited. For this reason, most of the currently available bioaccumulation models for IOCs in aquatic organisms do not explicitly address the potential role of membrane transporters/active transport.

ABSORPTION

Uptake across the gills

The passive absorption of an organic chemical at fish gills involves several sequential steps. A chemical is pumped through lamellar channels, diffuses to the epithelial surface, crosses multiple membranes, diffuses away from the epithelium into the blood, and becomes distributed among the various components of the blood (e.g., plasma, plasma proteins, red blood cells). As such, passive absorption depends on the rate at which the chemical is made available to the gills in ventilated water, the permeability of various aqueous and membrane layers, and the rate at which blood flow removes the chemical [63–71].

For IOCs, the different properties of the neutral and charged forms further complicate the situation. Because of the low membrane permeability of organic ions, increased ionization (i.e., fraction of the chemical in charged form) is expected to reduce gill absorption rates. However, Saarikoski et al. [72] noted that this decrease was not proportional to the extent of ionization in the exposure water and considered how the charged species may contribute to absorption. To address these potential mechanisms, Erickson et al. [31,32] modified a finite element model given previously by Erickson and McKim [70] for uptake of neutral organic compounds. The revised model accounts for the possibilities that 1) ions contribute to diffusion to and from membranes, which facilitates absorption by maintaining steep gradients of the neutral form across the membranes; 2) the pH at the gill surface is reduced compared with the bulk water pH as a result of elimination of metabolically derived acid; and 3) there is some permeation of ions through membranes, alone and/or in association with counter-ions.

The model given by Erickson et al. [31,32] explained important aspects of the effects of pH and buffering on gill absorption rates for several chlorinated phenols in large rainbow trout, but there were deviations of the data from model predictions indicative of the need for better understanding of partitioning and permeability of ionizable chemicals in biological tissues. A second version of the model, parameterized for fathead minnows, successfully described pH effects on

bioconcentration of diphenhydramine, a weakly basic pharmaceutical [73], in short-term waterborne exposures. The broader utility of this model is limited, however, by the need to specify about 20 parameters regarding fish morphology/physiology and chemical partitioning/permeability.

To more simply predict effects of ionization on gill absorption, Armitage et al. [11] modified the Gobas and Mackay [68] gill uptake equation for neutral compounds by adjusting the resistance to chemical transport in the organic phase based on the ratio of the total concentration to that of the neutral form, consistent with consideration 1 above (see Supplemental Data, Section S4 for details). Calculation of this ratio requires estimation of gill surface pH, consistent with the reduction of pH at the gill surface as a result of elimination of metabolically derived acid (i.e., consideration 2 above). In addition, the model accounts for direct absorption of ions across membranes via paracellular transport, which broadly addresses consideration 3 above.

A preliminary comparison of the gill models given by Armitage et al. [11] and Erickson et al. [31] was performed as part of the present work by simulating data given by Erickson et al. [31,32] for rainbow trout (*Oncorhynchus mykiss*) and Saarikoski et al. [72] for guppies (*Poecilia reticulata*). To perform this comparison, the Erickson et al. [31] model was used to predict the average pH at the gill surface, which was then used as an input to the Armitage et al. [11] model. Thus, in general, differences in predictions generated by the 2 models do not relate to differences in pH at the gill epithelium. The results of this model intercomparison are presented in the Supplemental Data (Section S5). Both models successfully describe data sets provided by Erickson et al. [31,32]. However, the model provided by Erickson et al. [31] performs better when simulating data from Saarikoski et al. [72], particularly at elevated pH (see Supplemental Data, Figure S1). The generally good performance of both models suggests that the presence of mucus at the gills is not an influential factor, at least for the IOCs considered.

Uptake in the gastrointestinal tract

As with uptake at the gills, passive uptake of organic chemicals in the gastrointestinal tract can be described using advective transport terms (i.e., the flow of digesta through the gastrointestinal tract and transport away from the gastrointestinal tract in blood) and diffusive transport terms (i.e., passive transport across aqueous and organic layers) [74,75]. Micelle-mediated transport across the unstirred water layer in the lumen has also been proposed as an important process facilitating uptake [76]. However, only the advective and passive transport processes are considered in most generic gut uptake models. A key factor distinguishing uptake across the gut wall from uptake at the gills is the residence time, which is on the order of hours in the gut compared with fractions of a second in gill lamellar channels. For neutral organic chemicals, reported chemical uptake efficiencies in the gut (E_D) tend to be fairly constant over a broad range of hydrophobicity (K_{OW}), with reduced uptake efficiencies observed only for very hydrophilic ($\log K_{OW} < 0$) and superhydrophobic ($\log K_{OW} > 7$–8) chemicals [74,75]. The reduction in chemical uptake efficiencies for very hydrophilic chemicals is a result of increased membrane resistance (i.e., diffusion across organic layers), whereas the transport terms for advection in the gut ("ingestion flow delay") and diffusion across aqueous layers become rate-limiting as hydrophobicity increases [74,75]. Biotransformation within the gastrointestinal tract may substantially reduce a compound's uptake efficiency regardless of its hydrophobicity [77–79].

Arnot and Quinn [80] recently published a critical review of dietary uptake studies of organic compounds in fish. Of the 400 observations, approximately 7% are for IOCs that are appreciably charged in the gut, including perfluorinated and polyfluorinated alkyl substances, pesticides (e.g., metconazole, fipronil), and industrial chemicals (e.g., pentachlorophenol). Reported or derived chemical uptake efficiencies for IOCs range from 0.7% to 125%, with the upper value clearly erroneous (100% being the maximum). Although there is considerable scatter and inconsistency in the data, there is no indication that uptake efficiencies of the IOCs are greatly reduced in comparison with neutral organic chemicals with similar properties (e.g., K_{OW}). For example, Xiao et al. [81] reported apparent and benchmarked chemical uptake efficiencies for pentachlorophenol, an organic acid ($\log K_{OW,N} = 5.1$, $pK_a = 4.7$), that were within 10% of values for pentachlorobenzene, a neutral compound ($\log K_{OW} = 5.2$). These results are consistent with the analysis of chemical uptake efficiencies of drugs in humans published by Abraham et al. [38], which suggested that there was no strong relationship between degree of speciation and uptake efficiency. Abraham et al. [38] further concluded that chemical uptake efficiency of IOCs could, as a first approximation, be reasonably estimated from properties of the neutral species. A similar conclusion was reached by O'Connor et al. [75]. In summary, uptake efficiencies of IOCs in the gut are not expected to be greatly reduced as a consequence of speciation unless the ratio of charged to neutral species is very large. Additional studies on the chemical uptake efficiencies of quaternary amine compounds or other highly dissociated IOCs (e.g., linear alkylbenzene sulfonates) would therefore be valuable.

Dermal uptake

For neutral organic compounds in water, the relative importance of dermal uptake compared with gill uptake is largely a function of the size of the organism. For example, dermal uptake may account for up to 50% of total uptake in waterborne exposures with smaller fish/early fish life stages (<4 g), whereas experiments with larger fish (500–1000 g) indicate only a minor contribution (≤10%) [82–84]. Factors accounting for the increased importance of dermal uptake in smaller fish include a larger skin/branchial surface area ratio, a larger skin surface area/body size ratio, and a thinner, more vascularized skin tissue (i.e., shorter diffusion distance). Dermal uptake rates also may depend to some extent on chemical solubility in the outermost layers of the skin, although in general this appears to be less true for fish than it is for mammals [84].

Empirical data characterizing the dermal uptake of IOCs are limited but suggest that this process can contribute to total uptake from the water phase, at least for smaller fish. Tovell et al. [85] estimated that dermal uptake accounted for approximately 20% of total absorption of sodium lauryl sulfate (an anionic surfactant) in goldfish (3.8–105 g), whereas Saarikoski et al. [72] reported that dermal uptake of pentachlorophenol ($pK_a = 4.7$) at pH 5 and 9 accounted for 24% and 38% of total absorption, respectively, in guppies (60–100 mg). The presence of negatively charged mucus does not appear to be a limiting factor in these cases.

DISTRIBUTION

The internal distribution of organic chemicals in biota is influenced by the rate of chemical delivery to tissues in blood,

transport across biological membranes separating blood from the interior of cells, and the relative affinity (sorption capacity) of different tissues for specific substances. For many neutral chemicals, passive diffusion is the dominant mechanism of membrane transport, and steady-state distribution may be adequately predicted from the total lipid content of individual tissues [86,87]. Under these conditions, lipid-normalized concentrations in different tissues will tend to the same value, as has been observed for hydrophobic chemicals of concern [88,89]. More complex approaches distinguish between different types of lipids and other biological macromolecules (e.g., storage lipids, phospholipids, plasma proteins, structural proteins) [90], but the conceptual approach and assumptions are essentially the same.

The internal distribution of IOCs is driven by the same basic considerations but complicated by the different behavior of charged and neutral species. For example, interactions between storage lipids (e.g., triglycerides) and charged compounds are not favorable [37]; hence, adipose is not an important reservoir for strongly dissociated IOCs (e.g., PFAAs) [91,92]. The key factors underlying the passive tissue distribution of IOCs are phospholipid content and plasma protein content [93–96]. Accordingly, tissues with relatively high phospholipid content (e.g., liver, kidney) and/or plasma protein content (e.g., blood) are expected to exhibit larger wet weight concentrations of IOCs, all else being equal. Acidic phospholipids (e.g., phosphatidylserine) will interact favorably only with bases, whereas zwitterionic (neutral) phospholipids (e.g., phosphatidylcholine) are important for both acids and bases [37,96,97]. Specific binding interactions that saturate at high substrate concentrations are also possible. This is particularly true for plasma binding of APIs that resemble ionized endogenous molecules. Weakly acidic drugs generally bind to albumin, whereas basic compounds bind to α_1-acid glycoprotein. Because this type of binding involves specific interactions with molecular binding sites, it is more difficult to predict from simple partitioning properties [98]. Fish are known to possess both albumin and α_1-acid glycoprotein; however, binding data for IOCs in fish plasma are scarce, and the potential variability between species is not well characterized.

An additional consideration for organic bases that are substantially charged at physiological pH (i.e., $pK_a > 8$) is lysosomal sequestration [99,100], which is an intracellular example of the general phenomenon known as the "ion trap" effect. Assuming the concentration of the neutral form in both fluids is equal, the large pH gradient between fluid within lysosomes (pH = 4–5) and cytoplasm (pH = 7.2) means that the concentration of the charged form of a monoprotic base could be approximately 2 to 3 orders of magnitude larger in the lysosome, assuming that pH-dependent partitioning holds. The potential effect is even larger for multiprotic bases. Partitioning to phospholipids within the lysosomes would also be greater relative to the cytoplasm [99]. Following from these considerations, cells with an abundance of lysosomes (e.g., liver, kidney, spleen, lung) have a greater inherent capacity to accumulate organic bases compared with tissues with relatively few lysosomes (e.g., muscle) [99,101]. Determining the relative importance of lysosomal sequestration compared with general sorption to phospholipids in vivo is challenging [99–101], and the implications for assessing the bioaccumulation potential of organic bases in aquatic organisms at the whole-body level are unclear. However, the results published by Daniel and Wojcikowski [101] suggest that, although important, lysosomal sequestration is secondary to general sorption to phospholipids in determining tissue partitioning, at least for the monoprotic bases and experimental conditions considered in that study.

Active transport mechanisms for membrane transport of IOCs could potentially result in tissue distribution patterns that differ from those predicted by simple partitioning considerations. However, we are not aware of any systematic studies in aquatic organisms that could be used to assess the potential importance of these processes in a generic manner. In practical terms, it may be difficult to distinguish the influence of active transport on internal distribution because of measurement uncertainties inherent to in vitro approaches used to derive tissue/water and tissue/plasma partition coefficients. For example, tissue/plasma distribution ratios predicted by Schmitt [96] using an equilibrium partitioning approach differed from measured values by a factor of approximately 1.5 to 5.0, depending on the tissue. Clearly, expectations based on simple equilibrium partitioning need to be better defined to inform discussions of the potential role of active transporters. Additional data characterizing the phospholipid and plasma protein content of tissues in aquatic organisms are needed, as are more data quantifying sorption affinities for IOCs (e.g., phospholipid/water distribution ratios and protein binding affinities). Such data can readily be incorporated into the existing mechanistic models that already distinguish among these different biological phases [11,33].

METABOLISM (BIOTRANSFORMATION)

Phase I biotransformation reactions, including those catalyzed by cytochrome P450 (CYP) monooxygenase enzymes, tend to increase a compound's polarity by adding or exposing a polar atom, whereas the major phase II pathways further enhance a compound's polarity by conjugation with a polar endogenous molecule. Acting alone or in concert, these pathways generally increase a compound's water solubility and may result in products that are substrates for specific membrane transporters.

In mammals, neutral compounds secreted to bile or filtered at the kidney tend to be reabsorbed. For this reason, most ionizable drugs possessing a log $D_{OW} > 1.0$ at pH 7.4 require biotransformation to facilitate their elimination from humans. Reabsorption in the liver and kidney also occurs in fish; however, fish can efficiently eliminate neutral compounds across the gills, provided that they exhibit only moderate hydrophobicity (log $K_{OW} < 3$). For such compounds, biotransformation is not required to facilitate elimination. Modeled effects of biotransformation on accumulation of IOCs by fish are presented as part of the gill uptake case study (see section *Case Study 1: The Relationship of the pH Dependence of BCFs to Gill Exchange Processes and Biotransformation*). This assessment is complicated by uncertainties relating to branchial flux of IOCs (e.g., pH changes at the gill surface). Nevertheless, biotransformation is predicted to have little impact on accumulation of an IOC that possesses moderate hydrophobicity as the neutral form (log $K_{OW,N} < 3$), unless the compound is very strongly dissociated.

Because of this fundamental difference between fish and mammals, it is possible that CYPs which transform xenobiotic substances have evolved somewhat differently in the 2 taxa. Based on sequence homology as well as functional studies, it is clear that fish possess counterparts to the CYPs responsible for metabolizing relatively hydrophobic compounds in mammals (primarily CYP1A and CYP3A forms). In contrast, fish do not

appear to possess orthologs for CYP2C9 and CYP2D6, both of which transform numerous ionizable drugs in humans [102,103]. Limited functional studies with prototypical CYP2C9 and CYP2D6 substrates also indicate that trout liver S9 fractions possess little (if any) activity toward these compounds [104].

Some IOCs are metabolized in fish by phase II conjugation reactions [102,105]. Existing studies indicate that in fish a number of resin acids and phenolic compounds undergo glucuronidation, whereas other work suggests that fish metabolize some carboxylic acids by amino acid conjugation. Sulfonation may also be a relevant process for certain acidic functional groups. In general, however, the role that these pathways play in limiting the accumulation of IOCs is poorly known. More research is needed to characterize the biotransformation of IOCs by fish, as this will help clarify the extent to which information from mammals can be "read across" (see section *Read-Across for Bioaccumulation/ADME*). The validation of in vitro test systems (e.g., liver S9 or hepatocytes) for IOCs would greatly facilitate such efforts [106]. Robust methods to extrapolate in vitro data for IOCs to the in vivo/whole-body situation are also required.

EXCRETION

Elimination across the gills

All current approaches used to model the absorption of IOCs at fish gills assume that uptake occurs by passive diffusion in response to a chemical activity gradient [11,31,32]. Because the process of passive diffusion is inherently nonsaturable and bidirectional, these models have equal utility for describing elimination. Active transport is not considered in available modeling approaches for gill exchange, and the potential relevance of such processes is discussed below (see section *Evidence for transporter-mediated chemical flux in the gills and gut*).

Fecal elimination

Passive elimination of organic chemicals through fecal egestion requires the diffusion of the chemical across the gut wall back into the lumen, where sorption to the remaining gut contents will occur. Some bioaccumulation models assume that transport terms describing gut uptake and elimination are equal, whereas others assume that processes such as micelle-mediated transport result in larger uptake rates compared with elimination [74]. The key drivers of fecal elimination are the chemical gradient between blood and feces (based on concentration and sorption capacity) and the efficiency with which the chemical can diffuse across the gut wall. Following from the discussion of the intestinal absorption, it is reasonable to assume that diffusive transport can be modeled as a first approximation using the properties of the neutral form. Again, IOCs that are very strongly dissociated could be exceptions and are therefore priorities for additional research.

Evidence for transporter-mediated chemical flux in the gills and gut

The ATP-binding cassette transporters are expressed in teleost gill tissue [107,108] and could contribute to branchial flux of specific chemical substrates. Stott et al. [109] employed a primary gill culture system to examine transport of 7 pharmaceuticals under various experimental conditions. The authors reported that there was some evidence for active transport; however, passive transport was the primary determinant of exchange across the interface (e.g., active transport accounted for approximately 10% of total transport for propranolol and imipramine). Additional studies indicate that transporters may play a role in the fish intestine similar to that in mammals, where multixenobiotic resistance pumps act as "gatekeepers" prohibiting uptake of certain compounds from food [107,108,110]. It is unclear whether any of these processes can substantially impact the accumulation of a xenobiotic substance in fish. Additional research is needed to determine whether active transport rates are comparatively faster than passive transport rates and, if so, to delineate the circumstances under which these differences are important for bioaccumulation assessment. Currently available mechanistic BCF models [11,31] do not account for active transport in the gill and gut. Accordingly, if significant active transport can be unambiguously demonstrated, predictive models that consider only passive flux at the gills and/or gut would have to be revised.

Biliary elimination

In mammals, SLC transporters present in the basolateral membrane of liver hepatocytes promote the uptake of organic anions from plasma, whereas ATP-binding cassette transporters in the canalicular membrane actively secrete organic anions, cations, and conjugated products of phase II biotransformation into bile [111–113]. Both of these processes play an important role in supporting the liver's normal biological functions (e.g., secretion of bile salts), and both have been shown to promote the elimination of ionized xenobiotics.

Although fish express a diverse array of membrane transporters in liver tissue, there have been relatively few studies demonstrating their functional role in the intact animal. Biliary secretion of phenol red has been studied in several species [114–116]. Hagfish, flounder, and catfish eliminated phenol red primarily as the parent compound; in trout, skates, and sharks, however, much of the phenol red was glucuronidated before its secretion to bile. Rainbow trout eliminated sulfobromophthalein to bile, both as the parent compound and as 1 or more conjugated products [117,118]. Indocyanine green was secreted to bile in hagfish, flounder, skates, and sharks [115]. Phenol red, sulfobromophthalein, and indocyanine green have been used extensively to study biliary secretion in mammals; and all 3 compounds exist predominantly as anions at physiological pH values.

Conjugates secreted to bile may be cleaved in the gastrointestinal tract by microbial enzymes, releasing the parent compound. If this compound is reabsorbed, the cycle can be repeated, resulting in a process called enterohepatic circulation. Limited studies indicate that enterohepatic circulation can occur in fish [119–121]; however, the toxicological significance of this process is largely unknown, and this process is not explicitly incorporated into currently available mechanistic models for aquatic organisms.

Renal elimination

In fish, as in mammals, the chemical content of urine reflects the net result of glomerular filtration, tubular secretion, and reabsorption [122,123]. Importantly, the urine flow rate of a fish is profoundly impacted by the environment within which it lives. In general, seawater-adjusted fish produce small quantities of relatively concentrated urine, whereas freshwater fish produce large volumes of dilute urine.

The urine has very little capacity for neutral hydrophobic organic chemicals relative to that of kidney tissue. For this reason, neutral hydrophobic compounds filtered at the

glomerulus are largely reabsorbed. In contrast, renal clearance may be an important route of elimination for some IOCs. Chemicals that are freely dissolved in blood plasma will be filtered at the glomerulus. Once in urine, these substances will dissociate in accordance with the local pH environment and, if they remain ionized, will tend to be retained in urine. James-Curtis and Wood [124] determined the pH of trout urine to be 7.3, which is somewhat lower than that of plasma (typically 7.8–7.9), and showed that this value could be adjusted upward by infusing animals with $NaHCO_3$. In humans, the pH of urine can be manipulated with acid (e.g., oral vitamin C) or base (infused $NaHCO_3$) to "trap" ionized drugs, facilitating their elimination, or to promote formation of the neutral form, causing them to be retained. We are not aware of any research demonstrating pH effects on renal clearance of IOCs by fish.

The glomerular filtration rate determined for trout is somewhat less than 1% of total blood flow to the kidney [125] and establishes an upper limit on renal clearance that can be achieved by simple filtration. For some IOCs, however, active secretion into urine can result in clearance rates that substantially exceed the glomerular filtration rate. This is because renal tubular secretion operates against the entire kidney blood flow. In addition, chemicals bound in plasma can desorb and become available to membrane transport proteins. Under these circumstances, the renal clearance of a chemical becomes rate-limited by blood flow to the kidney, which in trout approaches 60% of total cardiac output [126]. The potential significance of tubular secretion as a mechanism for chemical elimination was highlighted by the classic work of Pritchard and James [127], which showed that in winter flounder renal clearance of dichlorophenoxyacetic acid ($pK_a = 3.1$), a known substrate for OATs/Oats, was 500 times higher than the measured glomerular filtration rate. Additional whole-animal studies with fish have demonstrated renal clearance of 2,2-bis(p-chlorophenyl)-acetic acid, the acetic acid derivative of DDT [128], as well as ionized phase II conjugates of phenol [125] and several benzo[*a*]pyrene metabolites [129]. In a recent study with rainbow trout, Consoer et al. [130] found that urinary elimination of perfluorooctanoate (PFOA) substantially exceeded the rate predicted from glomerular filtration and was the primary route of chemical clearance. This finding is consistent with mammalian studies that show that membrane transporters (primarily OATs/Oats and OATPs/Oatps) control renal clearance of PFOA and are largely responsible for observed species differences in elimination half-life (see section *Case Study 2: Toxicokinetics of PFAAs*). Additional work with trout has suggested that perfluorooctane sulfonate (PFOS) is not secreted to urine [131]. Sulfonated PFAAs tend to accumulate in fish to a greater extent than carboxylated derivatives with the same number of perfluorinated carbons. One possible explanation for this finding is that the perfluorocarboxylates are much better substrates for membrane transporters in the fish kidney compared with perfluorinated sulfonates. Most currently available mechanistic BCF models do not account for renal clearance. Whereas passive elimination across the gills is expected to dominate over renal clearance occurring as a result of simple filtration, it is clear that active renal clearance can be a key elimination process for certain chemicals and therefore deserves further exploration.

READ-ACROSS FOR BIOACCUMULATION/ADME

Environmental risk assessors commonly "read across" existing information to untested conditions of interest. When applied to in vivo toxicity testing data, this approach assumes that different compounds sharing the same mode of action will have similar effects in the same or different species if their unbound concentration at the site of action is the same. This mode of action assumption implies, in turn, that relevant molecular targets are present in all species of interest and that the biological function of these targets is conserved. Because the unbound concentration at the site of action is determined by ADME considerations, use of the read-across approach to extrapolate among species requires consideration of the manner in which compounds are handled by different organisms.

With respect to predicting the effects of IOCs in fish, the read-across hypothesis articulated by Huggett et al. [22] is of special interest. This hypothesis states that a drug is likely to cause pharmacological effects in fish if the steady-state concentration in plasma approaches that associated with a therapeutic response in humans. To predict the drug concentration in plasma from that in water, Huggett et al. [22] employed an empirically based equation given by Fitzsimmons et al. [132]. This equation was derived using data for neutral organic chemicals and predicts blood (plasma)–water partitioning based on nonspecific interactions with organic constituents using a compound's log K_{OW} value. Several authors have adapted the Fitzsimmons et al. [132] equation for use with ionizable drugs by replacing log K_{OW} with the log D_{OW} calculated for an assumed pH value [23,133–135]. Unfortunately, this simple modification may result in underestimation of plasma binding because D_{OW} is not a representative surrogate for the interactions of IOCs with key biological macromolecules [37,73,98]. Experimental support for the read-across hypothesis proposed by Huggett et al. [22] has been provided by several studies [135,136]. To date, however, there have been very few studies with fish in which drug concentrations in plasma were measured and for which measured end points can be related to human therapeutic effects [137].

The accumulation of ionizable drugs by fish will be impacted by ADME processes as discussed throughout the present article. Development of a predictive capability for fish requires, therefore, that we learn how to read across not only therapeutic plasma levels but also basic ADME information from human drug development efforts. For some processes, such as plasma and tissue binding, it may be possible to quantitatively read across measured values. For example, Nichols et al. [73] found that the ionized form of diphenhydramine binds extensively in plasma of fathead minnows. Measured concentrations in whole animals also suggested a high level of tissue binding. These binding relationships could not have been predicted from nonspecific partitioning of neutral diphenhydramine. An examination of the drug literature suggested, however, that the apparent volume of distribution of diphenhydramine in fathead minnows (3 L/kg; calculated as the whole body-plasma concentration ratio) was similar to that in humans (3–8 L/kg) [138]. Had the authors adopted mammalian binding values as initial inputs to their steady-state model, they would have accurately predicted observed levels of accumulation.

For other processes, such as biotransformation or the involvement of membrane transporters, it is less likely that quantitative information from mammals can be read across to fish. Indeed, even among mammals there are large species differences in biotransformation (rates and predominant products) and the activities of different transporters. Nevertheless, it may be possible to use information from mammals to

identify processes of likely importance in fish and make qualitative predictions regarding their relative magnitude (e.g., fast, slow, and no metabolism). In a recent effort, Berninger et al. [139] compiled ADME data (therapeutic maximum concentration, whole-body clearance, volume of distribution, and half-life) for approximately 1000 drugs and used this information to rank the environmental hazard associated with these substances. Although not employed for this purpose, the values assembled by these authors could be combined with other information (e.g., pK_a and log $K_{OW,N}$) to assess the potential for individual drugs to accumulate in aquatic species.

CASE STUDY 1: THE RELATIONSHIP OF THE pH DEPENDENCE OF BCFs TO GILL EXCHANGE PROCESSES AND BIOTRANSFORMATION

Previous sections described how chemical properties, environmental variables, and gill physiology can affect uptake and elimination rates for IOCs at fish gills. However, the net accumulation of a chemical in a fish will usually be of more interest than these separate exchange rates. Also, the factors that determine this net accumulation might differ from those that impact the rates. For example, the contribution of ions to diffusive flux in aqueous phases will generally affect both uptake and elimination rates similarly, and thus affect the rate at which steady state is reached, but might be of little or no consequence to steady-state accumulation. The present case study addresses the pH dependence of steady-state accumulation and how this may be affected not only by processes controlling gill uptake and elimination but also by biotransformation of IOCs.

The present case study was not intended to provide a complete, definitive model for IOC bioaccumulation, to comprehensively compare existing models, or to assess model performance over a broad data set. Instead, we sought to identify and illustrate processes important to the pH dependence of IOC accumulation that warrant attention in further research and model development. Relevant predictions were obtained using the gill model of Erickson et al. [31], as implemented by Erickson et al. [32] and Nichols et al. [73] for trout and fathead minnows, respectively. In all cases, the steady-state blood:water bioconcentration factor (BCF_{BW}) was used to examine the dependence of IOC accumulation on external pH, because this is directly linked to the processes at the gill regulating this dependence. Absent specific mechanisms that could impact the internal distribution of a compound (e.g., membrane transporters operating in specific tissues and/or tissue-specific binding), whole-body accumulation is expected to be proportional to BCF_{BW} and thus to have the same pH dependence. To be consistent with current regulatory criteria for assessing bioaccumulation potential [19], the focus will be on the accumulation of the parent compound only.

Initially, we considered how IOC bioaccumulation reflects the processes affecting gill uptake (k_1) and elimination (k_2) rates. For 3 example chemicals, Figure 1 illustrates how BCF_{BW} varies with pH and compares these changes to pH-dependent changes in k_1 and k_2. Simulations and data are provided for 2 weak acids, 2,3,4-trichlorophenol and 2,3,4,5-tetrachlorophenol, for which k_1 and k_2 were measured for large rainbow trout at multiple bulk water pH values [32]. The BCF_{BW} values given in both panels were estimated as the ratio of k_1 to k_2. Additional simulations and data are given for a weak base, diphenhydramine, for which the BCF_{BW} was measured in fathead minnows [73].

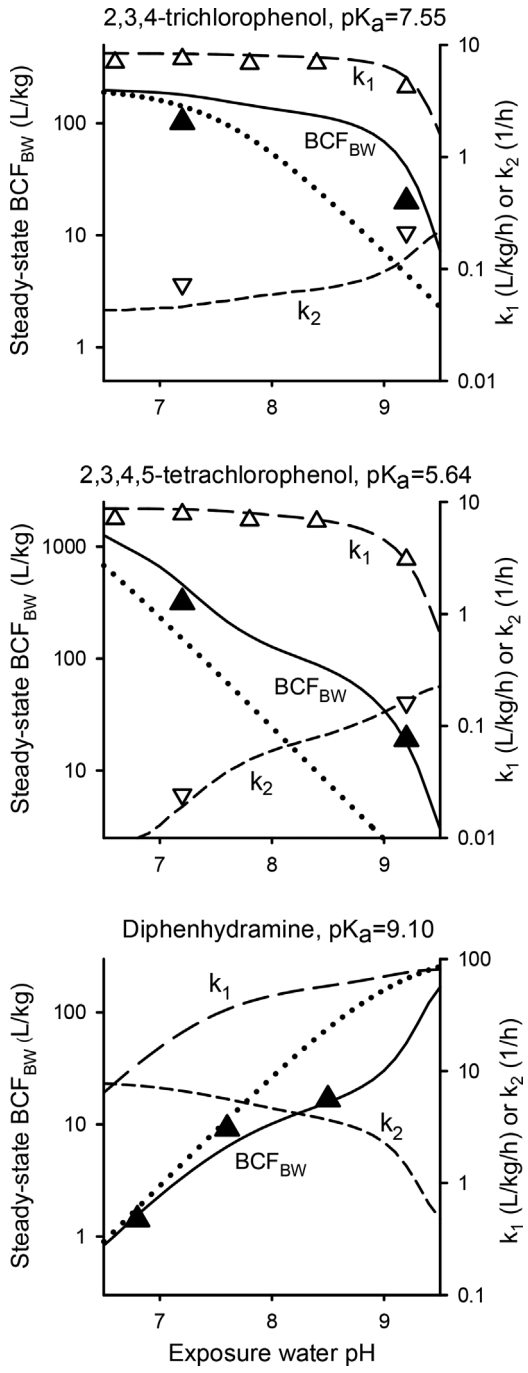

Figure 1. Model [31] predictions and observed values for the gill uptake rate constant (k_1; open upright triangles), the gill elimination rate constant (k_2; open inverted triangles), and the steady-state blood/water concentration factor (BCF_{BW}; closed upright triangles) as a function of pH for the weak acids 2,3,4-trichlorophenol (log octanol–water partition coefficient for the neutral form of the compound [$K_{OW,N}$] = 4.0) and 2,3,4,5-tetrachlorophenol (log $K_{OW,N}$ = 4.2) and the weak base diphenhydramine (log $K_{OW,N}$ = 3.6). Solid, long dashed, and short dashed lines show predicted values for BCF_{BW}, k_1, and k_2, respectively, where the modeled BCF_{BW} is the ratio of k_1 and k_2. Dotted line denotes what the model-calculated BCF_{BW} would be in the absence of any pH reduction in the gills. pK_a = dissociation constant.

The model-predicted BCF_{BW} values shown in Figure 1 agree well with measured values and can be understood in terms of the factors regulating k_1 and k_2 as presented in the section *Uptake across the gills*. For all 3 chemicals, the BCF_{BW} varies much more with pH than does either k_1 or k_2 because of k_1 decreasing and k_2 increasing with increased ionization. Also, for all

3 chemicals, there are large differences between modeled BCF_{BW} values (solid lines) and model simulations generated assuming no pH reduction at the gill surface (dotted lines). Generally, a reduction in gill pH tends to increase the bioaccumulation potential of weak acids and decrease the bioaccumulation potential of weak bases. These trends occur because the reduction in gill pH results in a larger fraction of an organic acid being present in neutral form at the gill surface compared with bulk water. The opposite pattern is predicted for organic bases.

For 2,3,4-trichlorophenol, the BCF_{BW} varies by less than 2-fold from pH 6.5 to 8.5 as a result of both k_1 and k_2 being fairly constant. The constancy of k_1 reflects efficient extraction of chemical even at rather high ionization in the exposure water because of the mechanisms discussed previously (see section *Uptake across the gills*). The constancy of k_2 is a result of the pH at the gill surface being near or below this chemical's pK_a so that the fraction of chemical that is ionized does not change greatly over this pH range. At pH >8.5, chemical ionization becomes large enough to substantially reduce k_1 and increase k_2, resulting in large decreases in the expected BCF_{BW}.

In contrast to 2,3,4-trichlorophenol, the BCF_{BW} for 2,3,4,5-tetrachlorophenol is predicted to vary more than 10-fold from pH 6.5 to 8.5. While k_1 is still roughly constant in this range and very close to that of 2,3,4-trichlorophenol, k_2 declines markedly with decreasing pH because of changes in ionization associated with this chemical's lower pK_a value. The predicted decline in k_1 at higher pH values is moderately greater than that for 2,3,4-trichlorophenol, but the combined effects of k_1 and k_2 on the BCF_{BW} above pH 9 are similar for the 2 chemicals. Because of the limited number of measurements for k_2, the modeled shape of the BCF_{BW} versus pH curve is not empirically established; but it is clear that the effect of pH is greater for 2,3,4,5-tetrachlorophenol than for 2,3,4-trichlorophenol, consistent with model predictions.

As for the weak acids, the modeled k_1 for diphenhydramine (a weak base) decreases with increasing ionization in a less-than-proportional manner; however, this lack of proportionality is less pronounced than for the weak acids because pH reductions at the gill increase ionization for this weak base. The lower gill pH is also responsible for relatively small changes in the modeled k_2 at pH <9 because chemical at the lamellar surface is >90% ionized over this entire range. The modeled and measured BCF_{BW} values agree well, and both show a large deviation above pH 8 from the BCF_{BW} expected in the absence of pH reductions in the gill.

Next, we considered possible impacts of biotransformation on the pH dependence of IOC bioaccumulation. This effort was not intended to provide predictions about biotransformation rates as a function of pK_a, hydrophobicity, and other chemical properties but rather to consider whether the pH dependence of IOC bioaccumulation should differ for chemicals that have significant biotransformation rates from those chemicals that have negligible biotransformation. The influence of biotransformation on steady-state accumulation depends on the magnitude of the whole-body biotransformation rate constant (k_M) relative to k_2, such that for the same k_M biotransformation for non-ionizable chemicals becomes more important with increased chemical hydrophobicity (because of smaller k_2). Because ionization can affect k_2 (Figure 1), this raises the question of whether biotransformation would change the pH dependence of the BCF_{BW} for IOCs and be more or less important than it is for non-ionizable chemicals with the same hydrophobicity as the neutral forms of IOCs. There are no empirical data to directly address this question, so the calculations are hypothetical; however, the relative magnitudes of k_M and k_2 will govern the consequences of biotransformation, and this exercise can define its likely impact on the pH dependence of IOC accumulation.

Figure 2 illustrates how pH effects on BCF_{BW} would be expected to change as a result of biotransformation. These simulations were generated with the same model that was used to obtain Figure 1 (parameterized for trout [32]). In this case, however, predictions were obtained for 4 hypothetical chemicals with log K_{OW} of 3 and 5 for the neutral form, 2 being weak acids, with $pK_a = 6$, and the other 2 being weak bases, with $pK_a = 9$. The figure compares BCF_{BW} values predicted in the absence of biotransformation with those expected assuming moderate (0.02/h) and fast (0.1/h) rates of biotransformation. Because the purpose of this exercise was to illustrate the impact of a total rate of biotransformation on accumulation, there was no need to consider how biotransformation is affected by internal ionization or partitioning; however, this biotransformation must be considered to occur in tissues other than the gills because biotransformation in the gills [140–144] would directly impact apparent k_1 and k_2 values. A model developed to describe the effects of gill metabolism on chemical accumulation would therefore require modification of the gill description itself.

For the organic acid with log $K_{OW,N} = 3$, setting k_M to 0.1/h reduces accumulation compared with no biotransformation by a

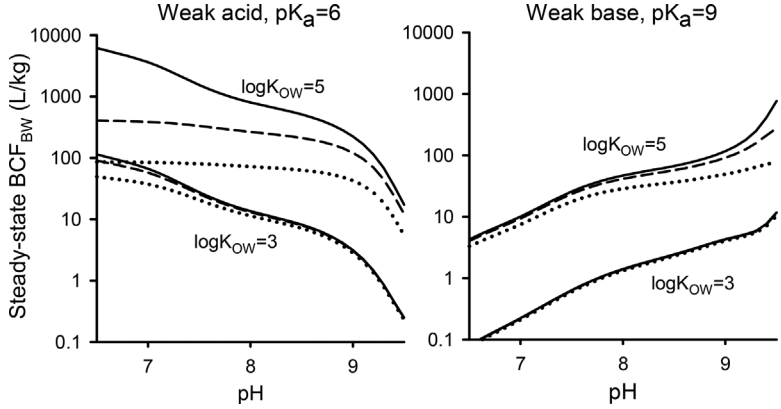

Figure 2. Modeled [31] effects of biotransformation on steady-state blood/water bioconcentration factors for hypothetical weak acids (left panel) and weak bases (right panel). Solid lines indicate no biotransformation; dashed lines indicate moderate (0.02/h) biotransformation; dotted lines indicate fast (0.1/h) biotransformation. BCF_{BW} = blood/water bioconcentration factor; K_{OW} = octanol–water partition coefficient; pK_a = dissociation constant.

factor of 3.0 at pH 6 (where k_2 is 0.05/h) but only by 7% at pH 9 (where k_2 is 1.0/h), thereby reducing the total variation of accumulation across pH. Using the same model, a non-ionizable chemical with log $K_{OW} = 3$ would have a $k_2 = 0.45$/h across the entire range of pH values and a $k_M = 0.1$/h would reduce the BCF_{BW} by 18%. Thus, not only should biotransformation alter the pH dependence of the bioaccumulation potential of acidic IOCs but also, depending on pH, the relative importance of biotransformation could be more or less than it is for a non-ionizable chemical of similar hydrophobicity.

The dampening effect of biotransformation on the pH dependence of BCF_{BW} becomes very evident for the more hydrophobic organic acid (log $K_{OW,N} = 5$) in Figure 2. When k_M is set equal to 0.02/h, the change in predicted BCF_{BW} over the pH range of 6 to 9 becomes relatively small because the biotransformation rate constant dominates elimination and the pH dependence of k_2 is less relevant. This effect is even more pronounced at the larger k_M of 0.1/h. Setting k_M equal to 0.02/h reduces the BCF_{BW} by a factor of 20 and 1.7 at pH 6 and 9, respectively, compared with simulations assuming no biotransformation. For a non-ionizable chemical with a log $K_{OW} = 5$, setting k_M to 0.02/h is predicted to reduce the BCF_{BW} by a factor of 5.0.

The model output for the 2 hypothetical bases indicates that, under the model assumptions, biotransformation will perturb the pH dependence of the BCF_{BW} less so than for the organic acids. For example, in Figure 2, biotransformation has a negligible influence on the pH dependence of the BCF_{BW} of the hypothetical base with log $K_{OW} = 3$. This pattern is observed because the gill elimination rate constants for these bases are much larger than the assumed biotransformation rate constants across the entire pH range. Biotransformation exerts a more substantial influence on the model output for the more hydrophobic base (log $K_{OW} = 5$), particularly at elevated bulk water pH (>8). This occurs because, as bulk water pH increases, the predicted gill elimination rate constant decreases (Figure 1) to the point where it is similar to the assumed biotransformation rate constant. In summary, because the relative impact of biotransformation on overall bioaccumulation increases as the rates from other elimination processes decrease, it is expected that the relative effect of pH on IOC accumulation will change as biotransformation rates change and thus be an important aspect of interpreting data on the pH dependence of IOC accumulation.

The present case study highlights some important needs for better assessing the accumulation and effects of IOCs. First, although there are considerable data on the bioaccumulation of various IOCs [11,25,145], little of this systematically addresses the effects of pH for the same chemical and species. However, from both collective data on IOC accumulation and specific data sets addressing pH effects, the general trend is clear: the bioaccumulation potential of organic acids is inversely correlated with bulk water pH, and the opposite is true for organic bases but not in proportion to the extent of ionization in exposure water. These trends can be related to various processes at the gills, but better data are needed regarding this to develop more rigorous models. Second, a critical factor in predicting the effect of pH on accumulation of IOCs is the reduction in pH at the gill surface, which can vary depending on ambient pH and buffering strength, as well as the organism's morphology and physiology. More information on this pH reduction under laboratory and realistic field conditions is greatly needed. This could be obtained by direct measurement or by inference, based on the dependence of accumulation on pH and pK_a. Third, many IOCs are subject to biotransformation, which can affect the pH dependence of the bioaccumulation potential of the parent compound. Studies that examine pH effects on accumulation could be combined with independently obtained estimates of k_M (e.g., by extrapolating biotransformation rate constant data from an in vitro system [146]) to corroborate modeled predictions.

CASE STUDY 2: TOXICOKINETICS OF PFAAs

Perfluorinated alkyl acids can be considered "extreme" IOCs in the sense that they have very low pK_a values (<1) and hence are >99% ionized at environmentally relevant pH values [147,148]. Nevertheless, field and laboratory studies have shown that long-chain PFAAs (i.e., those with at least 8 perfluorinated carbons) can accumulate in fish to the same extent as some neutral hydrophobic compounds (e.g., BCFs and BAFs on the order of 10^3 to 10^4, similar to mid-chlorinated polychlorinated biphenyls) [149].

Studies with both fish and mammals indicate that, with respect to chemical concentration, PFAAs accumulate preferentially in blood and liver [91,150,151]. In vitro studies have shown that PFAAs bind strongly to serum albumin [152–156] and liver fatty acid binding protein [157–159], which may at least partly explain their tissue-specific accumulation. Elimination half-lives of PFAAs vary widely among species and for some, such as the rat, between genders [160–163]. In mammals, this elimination is largely controlled by the rate of renal clearance, which has been linked to the interactions of PFAAs with membrane transport proteins. Cellular uptake and competitive inhibition studies indicate that membrane transport of several long-chain PFAAs can be mediated by OATs/Oats and OATPs/Oatps [164,165]. Some of these transporters appear to secrete PFAAs to urine, whereas others promote their reabsorption. For any given compound and species, the rate of renal elimination may reflect a balance of these 2 competing processes [162].

Based on these observations, Ng and Hungerbühler [33] developed a model to predict the bioaccumulation of PFAAs in fish. This model explicitly describes the interactions of PFAAs with serum albumin, liver fatty acid binding protein in liver cytosol, and membrane transporters in the kidney. Although the model can be adapted to different species, it was parameterized to predict BCFs and patterns of tissue distribution for several PFAAs in rainbow trout (*O. mykiss*) to compare predicted values with data from an earlier study by Martin et al. [91].

At the time of its development, few data existed for the interactions of PFAAs with fish plasma proteins and none for fish-specific liver fatty acid binding protein. There were also no studies that had looked specifically at renal elimination of these chemicals in fish. Binding to fish plasma proteins had been observed [166], however, whereas other studies had shown that fish possess Oats and Oatps and that these proteins transport a variety of organic anions (see section *Potential role of membrane transporters/active transport*). The model therefore used existing data from studies with rat and human proteins to parameterize protein binding interactions and renal clearance by membrane transporters in the trout kidney.

The recent publication of 2 detailed kinetic studies [130,131] presents an opportunity to test how model predictions based on protein interactions derived from mammalian systems compare with observed rates of uptake and elimination for PFOA and PFOS. Consoer et al. [130,131] used large trout confined to respirometer-metabolism chambers to directly determine renal and branchial clearance rates for both compounds, injected as a bolus dose. Additional studies were then performed to estimate

gill uptake rates during continuous waterborne exposures. We summarize our efforts to simulate the results of these studies using the model given by Ng and Hungerbühler [33]. Adjustments to the original model required to perform these comparisons, along with a complete description of modeled results, are presented in the Supplemental Data (Section S6).

In comparison with the experimental results of Consoer et al. [130,131], the original model overestimated measured rates of gill uptake (k_1) and elimination (k_2) for both PFOA and PFOS. A better match between the measured and predicted values was obtained by adjusting gill permeability constants downward by a factor of 15 (Table 1). The total elimination half-life ($T_{1/2}$) value for PFOA (2.67 d) predicted by the adjusted model using the median observed binding affinity constant (K_A) for albumin is one-fifth that determined by Consoer et al. (12.6 d) [130]. However, 12.6 d is well within the range of values predicted using all available albumin binding data (2–43 d). For PFOS, the predicted $T_{1/2}$ using the median K_A (68.7 d) is very close to that determined by Consoer et al. (86.9 d) [131]. Renal elimination rates (k_R) predicted by both the original and adjusted models were in good agreement with reported values. Thus, renal clearance appears to have been well-described using information derived from studies on mammalian cell lines.

Understanding the bioaccumulation of chemicals that deviate from expected behavior requires that we capitalize on existing toxicokinetic data and better understand the strengths and limitations of read-across approaches as they apply to bioaccumulation mechanisms. In the present case study, a convincing argument can be made for the ability to read across certain ADME processes from mammals to fish; that is, protein binding and active transport–mediated renal elimination were identified as key processes in mammals, and the comparisons we have made indicate that these processes are also important in rainbow trout. Some of the parameters derived using mammalian cell lines and proteins (e.g., binding affinities and active transport rates) worked well within the trout model, whereas others (e.g., equating the permeability of mammalian cells to gill permeability) were less successful. Given the importance of protein interactions to the toxicokinetics of PFAAs as a chemical class, we see the need for a better understanding of transporter-mediated uptake and elimination mechanisms in different organisms.

SUMMARY AND RECOMMENDATIONS

Several key conclusions stem from the present review:

Uptake and accumulation of IOCs from water may vary substantially with pH. Factors that influence these relationships include the identity of the compound itself (acid or base), its physicochemical properties (pK_a and log $K_{OW,N}$), and pH reductions at the gill surface as a result of elimination of metabolically derived acid. Existing gill models describe observed patterns with respect to pH effects on accumulation; however, data are needed to further refine these models. Active transport and biotransformation at the gills are not explicitly included in currently available mechanistic models. The relative importance of these processes remains poorly known.

Generally, pH effects on uptake and accumulation from water are predicted to be minor unless the extent of ionization in bulk water exceeds 90%. When the extent of ionization is <90%, models that consider only the hydrophobicity of the neutral form may be sufficient to describe accumulation.

Biotransformation is predicted to have a dampening effect on the pH dependence of bioconcentration for IOCs; however, the relative importance of biotransformation compared with gill elimination may differ substantially depending on whether the compound is an acid or base, as well as the hydrophobicity of the neutral form.

Biotransformation is expected to play a crucial role in the bioaccumulation of IOCs with large log $K_{OW,N}$ values. As with neutral organics, there are few measured biotransformation rates for IOCs in fish and other ecological receptors. Integrated testing strategies (e.g., in vitro studies with S9 [106] or hepatocytes) should be developed to strategically select IOCs for measurement of biotransformation rates in aquatic species.

For many (if not most) IOCs, uptake efficiency from the diet is not expected to be significantly impacted by the extent of ionization and can be adequately predicted from log $K_{OW,N}$. Active transport and biotransformation in the gut are not explicitly included in currently available mechanistic models but could potentially be addressed by adjusting the chemical uptake efficiency (E_D) estimated using current approaches [11,167].

For many IOCs, distribution to tissues is determined by nonspecific sorption to phospholipids and may be predicted using relatively simple models. By comparison, plasma binding of IOCs is more difficult to predict and more likely to involve specific interactions with transporter proteins such as albumin and α_1-acid glycoprotein. In general, binding interactions of IOCs with plasma proteins derived from fish are poorly known.

For some IOCs, we can expect interactions with proteins that transport ionized substances across biological membranes. In such cases, biliary or renal elimination may control the overall rate of elimination. Efflux transporters in the gut (and possibly gills) have the potential to limit uptake from the diet

Table 1. Measured and modeled kinetic constants for perfluorooctanoate and perfluorooctane sulfonate in large rainbow trout

			Ng and Hungerbühler [33],[a] adjusted[b]	
	Consoer et al. [130] PFOA mean (SD)	Consoer et al. [131] PFOS mean (SD)	PFOA median[c] (range)	PFOS median[c] (range)
k_1 (L/kg/d)	0.19 (0.09)	0.85 (0.29)	0.126 (0.03–0.46)	1.75 (0.19–2.43)
k_2 (1/d)	0.030 (0.011)	0.027 (0.010)	0.010 (0.0085–0.014)	0.014 (0.014–0.092)
k_R (1/d)	0.330 (0.236)	0.007 (0.003)	0.25 (0.024–0.33)	0.023 (0.023–0.33)
$T_{1/2}$[d]	12.6 (7.2)	86.9 (14.1)	2.67 (2.02–43.3)	68.7 (2.01–184)

[a]Model parameterized for a 1.1-kg rainbow trout using the urine flow rate given by Consoer et al. [130].
[b]Adjusted by reducing gill permeability by a factor of 15.
[c]Model predictions obtained using median, maximum, and minimum reported values for equilibrium association constants with albumin.
[d]Half-lives as reported by Consoer et al. [130,131]; for model predictions, these were calculated based on overall clearance rates.
PFOA = perfluorooctanoate; PFOS = perfluorooctane sulfonate; SD = standard deviation; k_1 = gill uptake rate constant; k_2 = gill (branchial) elimination rate constant; k_R = renal elimination rate constant; $T_{1/2}$ = whole-body elimination half-life.

(and possibly water). Although fish are known to possess a diverse array of transporters, there have been few studies evaluating their role as determinants of whole-animal chemical accumulation. Additional studies employing likely substrates for these transporters are needed. Currently, the potential importance of membrane transporters for each IOC must be assessed on a case-by-case basis.

Read-across approaches that employ information from mammals to inform ADME in fish may be useful for predicting the distribution of IOCs. This is especially true for ionized pharmaceuticals, which tend to be data-rich compounds. Limited data suggest that information pertaining to distribution (e.g., tissue and plasma binding) is most amenable to this approach. Information on metabolism and excretion is likely to be useful for identifying important processes in fish (e.g., the possibility of biotransformation or a likely role for membrane transporters) and may in some cases provide quantitative estimates of critical modeling parameters (e.g., renal tubular secretion of PFAAs).

Based on the foregoing and recognizing that there are regulatory demands to make decisions in the present, we recommend that interim methods for bioaccumulation assessment of IOCs follow the tiered approach outlined in Armitage et al. [11] using an appropriate bioaccumulation model (Figure 3). The workflow shown in Figure 3 is appropriate for a bioaccumulation hazard assessment prior to the generation of, or in the absence of, sufficient empirical data; however, the same approach could be applied when parameterizing models for exposure and risk assessment. For exposure and risk assessment, the decision-making context to potentially proceed to additional tiers would be driven by risk estimates (or margin of safety/exposure) rather than bioaccumulation assessment criteria. The general strategy is to initially use conservative assumptions and progress to more realistic and sophisticated assumptions (models and associated data requirements) as required. In tier 0, the IOC is treated as though it is a neutral compound and biotransformation is assumed to be negligible. Tier 1 incorporates biotransformation rate estimates from quantitative structure–activity relationship modeling (if within the applicability domain of the model) and/or from in vitro to in vivo extrapolation of measured biotransformation rates. Tier 2 incorporates refined gill uptake and elimination models and may consider site-specific exposure conditions (e.g., pH and water hardness). Empirical or in silico data characterizing the sorption behavior of the IOCs of interest are used directly in tier 2 but can also be used to complement assessments in lower tiers. The need for additional refinements would be informed by supporting test information (e.g., in vitro data) and/or existing ADME information from mammals, especially data indicating the importance of membrane/active transporters. Even if in vitro and ADME data from mammals cannot be used quantitatively in the bioaccumulation or exposure assessment, they can still serve as a qualitative flag indicating that uncertainty in the assessment is greater than for other chemicals. As discussed in the present study and elsewhere [145], the bioaccumulation potential of acidic IOCs is inversely related to pH, whereas it is directly related for basic IOCs. Accordingly, tier 2 bioaccumulation hazard assessments for acidic IOCs and basic IOCs should be conducted at pH 8.1 for marine systems and at the lowest and highest relevant bulk water pH values, respectively, for freshwater systems. Following from this, bulk water pH and test water characteristics (alkalinity, hardness) should always be reported and used to interpret in vivo bioaccumulation data used for B categorization. In this context, it is noteworthy that bulk water pH was reported for less than 40% of the BCF tests for the IOCs compiled by Arnot and Gobas [24]. Generic exposure and risk assessments should also be conducted across the relevant range of pHs, whereas site-specific assessments should be based on knowledge of the local conditions.

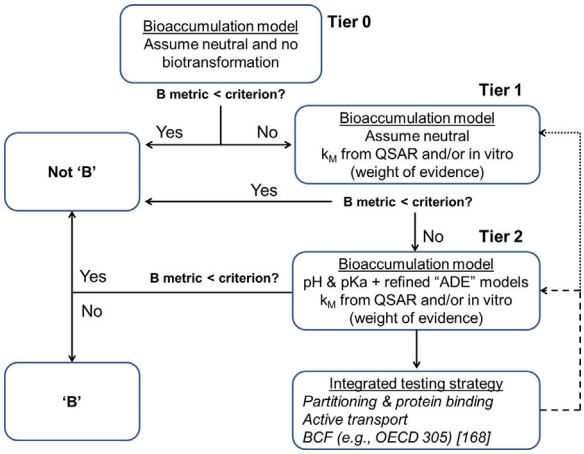

Figure 3. Proposed interim workflow for screening and prioritization of ionizable organic compounds for bioaccumulation hazard assessment and ecological exposure and risk assessment. For the latter, the "B question" would be replaced by the results and decision contexts for the exposure and risk estimates. ADE = absorption, distribution, and excretion; BCF = bioconcentration factor; k_M = whole-body biotransformation rate constant; OECD = Organisation for Economic Co-operation and Development; pK_a = dissociation constant; QSAR = quantitative structure–activity relationship.

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Data Availability—Modeling tools applied in the current review are based on publications in *Environmental Toxicology and Chemistry* and *Environmental Science and Technology*, as indicated. See publications on modeling tools identified in the review for data.

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