Critical Review

Recommendations for Improving Methods and Models for Aquatic Hazard Assessment of Ionizable Organic Chemicals

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Abstract: Ionizable organic chemicals (IOCs) such as organic acids and bases are an important substance class requiring aquatic hazard evaluation. Although the aquatic toxicity of IOCs is highly dependent on the water pH, many toxicity studies in the literature cannot be interpreted because pH was not reported or not kept constant during the experiment, calling for an adaptation and improvement of testing guidelines. The modulating influence of pH on toxicity is mainly caused by pH-dependent uptake and bioaccumulation of IOCs, which can be described by ion-trapping and toxicokinetic models. The internal effect concentrations of IOCs were found to be independent of the external pH because of organisms’ and cells’ ability to maintain a stable internal pH milieu. If the external pH is close to the internal pH, existing quantitative structure–activity relationships (QSARs) for neutral organics can be adapted by substituting the octanol–water partition coefficient by the ionization-corrected liposome–water distribution ratio as the hydrophobicity descriptor, demonstrated by modification of the target lipid model. Charged, zwitterionic and neutral species of an IOC can all contribute to observed toxicity, either through concentration-additive mixture effects or by interaction of different species, as is the case for uncoupling of mitochondrial respiration. For specifically acting IOCs, we recommend a 2-step screening procedure with ion-trapping/QSAR models used to predict the baseline toxicity, followed by adjustment using the toxic ratio derived from in vitro systems. Receptor- or plasma-binding models also show promise for elucidating IOC toxicity. The present review is intended to help demystify the ecotoxicity of IOCs and provide recommendations for their hazard and risk assessment. Environ Toxicol Chem 2020;39:269–286. © 2019 The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals, Inc. on behalf of SETAC.

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INTRODUCTION

Ionizable organic compounds (IOCs) are chemicals that are present in 2 or more forms (species) in the aquatic environment. They represent an important class of substances comprising almost 80% of orally ingested pharmaceuticals, the majority of which are monoprotic acids and bases carrying one acid or base function but including single and complex ampholytes (Manallack et al. 2013). They are also estimated to constitute 30 to 40% of industrial chemicals (Franco et al. 2010; Arp et al. 2017). Many pesticides are acidic or multiprotic, such as the phenoxyacetic acids, tinorganic compounds, and glyphosate.

Because standard methods often cannot directly be applied to IOCs, IOCs pose a challenge for aquatic hazard assessment (Franco et al. 2010). This issue concerns both testing guidelines that are optimized for effect assessment of neutral molecules and toxicity prediction models, for which the applicability domain rarely extends to IOCs.

Several studies have assessed the pH-dependent toxicity of IOCs, working typically in buffer-fortified test media to assure
control of pH (Rendal et al. 2011b). These studies show that the toxicity generally increased with the fraction of neutral species. These findings were interpreted with different models, often concluding that the neutral species is more toxic than the charged species.

Our first objective was to review the literature on IOC toxicity and then discuss improvements to testing strategies. We then describe different approaches to predict the aquatic toxicity of IOCs at ambient pH and as a function of pH. Because both bioaccumulation and toxicity typically increase with increasing fraction of neutral species, we argue that the pH dependence of toxicity is a result of the differences in bioaccumulation and toxicokinetics. Most organisms have well-buffered constant internal pH values, leading to constant internal effect concentrations so that the pH dependence of toxicity is effectively attributable to a pH-dependent uptake into organisms. Existing literature data will be reevaluated in the light of this hypothesis.

ENVIRONMENTALLY RELEVANT IOCs

Ionizable organic compounds are mono- or multirprotic acids or bases that are present in different molecular forms (species). The external pH in the bulk aqueous media, together with the acid dissociation constant, pKα, determine the fraction α of species i, to which the test organism is exposed, as given by Equation 1 for acidic IOCs (HA), α_{HA}, and for bases (BH^−), α_{BH^+}. The fractions of conjugated base (A for an acid and B for a base) are calculated by Equation 2. Given mass balance constraints, the sum of neutral and charged species for a monoprotic acid or base must equal 1 (Equation 3).

\[
\text{Fraction acidic species: } \alpha_{HA} \text{ or } \alpha_{BH^+} = \frac{1}{1 + 10^{pK_a - pH}} \tag{1}
\]

\[
\text{Fraction conjugated base: } \alpha_A \text{ or } \alpha_B = \frac{1}{1 + 10^{pK_a - pH}} \tag{2}
\]

\[
\text{Acids: } \alpha_{HA} + \alpha_A = 1 \text{ and bases: } \alpha_{BH^+} + \alpha_B = 1 \tag{3}
\]

The corresponding equations for diprotic acids and bases are derived from Zarfl et al. (2008) and Baumer et al. (2017), and more complex speciation can be predicted with SPARC (Hilal et al. 1995). A general estimation function for fractions of different molecular species is derived in the Supplemental Data.

In Figure 1 the experimentally accessible pH range (more details in the section Testing guidelines) is plotted as a function of the fraction of neutral species for monoprotic acids (Figure 1A) and bases (Figure 1B). Acids with pKα values >11 and bases with pKb values <4 are mainly present in their neutral form. In the experimentally accessible pH range of 5.5 to 9.5 (exact range depends on the biological organism), acids and bases with pKα values approximately from 5 to 10 occur in 2 forms, and speciation has to be accounted for in toxicity assessment.

Although speciation may be very complex, most of the available literature studies on the ecotoxicity of IOCs have focused on monoprotic acids and bases (Table 1). Most common bases are aliphatic amines that cover a pKα range of 7 to 11, but speciation may also be relevant for anilines (pKα 1–6) and heterocyclic aromatic nitrogen compounds (pKα 2–8; Schwarzenbach et al. 2016). For acids, speciation is relevant for phenols (pKα 2–9.6; Schwarzenbach et al. 2016), especially those with electron-withdrawing groups (e.g., pentachlorophenol pKα 4.75 or 2,4-dinitrophenol pKα 3.94; Escher et al. 2000). Saturated alcohols typically have higher pKα and are predominantly neutral in the environment (Schwarzenbach et al. 2016). Saturated thiacetals have pKα typically >8, but aromatic thiols have pKα values of 8 and lower (Schwarzenbach et al. 2016). Carboxylic acids are mainly charged at the pH values accessible for toxicity testing (pKα 3–4) but with electron-donating substituents may have pKα values up to 5 (Schwarzenbach et al. 2016). Benzoic and sulfonic acids are typically very acidic and fully deprotonated at the accessible pH range, so they are not IOCs but rather occur as fully charged species (Schwarzenbach et al. 2016).

Amphoteries (also called “polyprotic” acids and bases) have multiple acid and base functions and hence may undergo complex speciation across the experimentally accessible pH range. Of particular interest are those that form zwitterions that contain both positively and negatively charged moieties at ambient pH values. Examples include the antihistamine cetirizine, which has 3 acid/base-functions with the environmentally relevant pKα of 8 for the reaction from zwitterion to anion...
TABLE 1: Examples of acids and bases and their pKa ranges*

| IOC type       | Chemical class                      | pKa range | Example(s)                  |
|---------------|------------------------------------|-----------|------------------------------|
| Bases         | Aliphatic amines                    | 7–11      | Propranolol (pKa = 8.99)     |
|               | Anilines                           | 1–6       | 4-Chloroaniline (pKa = 3.99) |
|               | Heterocyclic aromatic nitrogens     | 2–8       | Pyridine (pKa = 5.25)        |
| Acids         | Phenols                            | 2–9.6     | Pentachlorophenol (pKa = 4.75)|
|               | Saturated alcohols                  | >14       | Ethanol, predominantly neutral at accessible pH range |
|               | Saturated thio-alcohols             | >8        | Ethanol thiolic (pKa = 10.6) |
|               | Aromatic thioles                    | <8        | Thiophenol (pKa = 6.5)       |
|               | Carboxylic acids                    | 3–4; may be as high as 5 with electron donating substituents |
|               | Benzoic acids                       | 3–5       | Ibuprofen (pKa = 4.45)       |
| Sulfonic acids | Fully deprotonated at accessible pH range | p-Toluenesulfonic acid (pKa = 0.70) |

*Adapted from Schwarzenbach et al. (2016).
IOC = Ionizable organic chemical.

(Baumer et al. 2017). Drugs such as sartans (e.g., telmisartan or valsartan), labetalol or enalapril also show a very complex speciation behavior with the acid and base functions so close together that even 3 species have to be simultaneously considered at a given pH (Baumer et al. 2017). The phytoestrogen genistein has 2 phenolic hydroxy groups with pKa values of 7.2 and 10, going from neutral to mono-anion to di-anion in the experimentally accessible pH range (Baumer et al. 2017).

One cannot rely on toxicity data tested at one pH value (even if that is well defined) because the pH of surface freshwater varies. Although typically in the range of pH 7 to 8, freshwaters may vary by up to 6 pH units (Boström and Berglund 2015). Hence, for the present review, we focus on studies that explored the pH dependence of toxicity or investigated many different chemicals with diverse speciation at one defined pH value.

PH DEPENDENCE OF AQUATIC TOXICITY OF IOCs

Testing guidelines

For the testing of IOCs, a stable and defined pH throughout the experiment is an essential prerequisite for the success of the study, but testing guidelines were often not developed with consideration of IOCs. A brief summary of specifications for different current aquatic toxicity tests and species is shown in Table 2. The given pH tolerance ranges might be reduced for chronic assays because of the longer exposure duration.

For algal toxicity, the pH is typically buffered by CO2 with the goal of maintaining the pH within 0.5 pH units during the test according to Organisation for Economic Co-operation and Development (OECD) guideline 201 (Organisation for Economic Co-operation and Development 1984).

The growth rate of the controls in the algal toxicity assay performed according to OECD guideline 201 (Organisation for Economic Co-operation and Development 1984) with additional 5 mM buffer (bis-tris propane) already varied in the controls, but it was possible to derive robust effect concentration (EC) values for triclosan at pH 7, 8 and 8.5 (Roberts et al. 2014).

The acute toxicity assay for Daphnia magna is normally carried out according to OECD guideline 202 (Organisation for Economic Co-operation and Development 2004) in water without adjustment of pH apart from adjustment of the pH in the stock solution if the pH drifts beyond a range of 6 to 9 (Rendal et al. 2012). For the chronic test with D. magna, pH 6 to 9 is acceptable according to OECD test guideline 211 (Organisation for Economic Co-operation and Development 2012).

The revised OECD test guideline 203 for fish acute toxicity testing (Organisation for Economic Co-operation and Development 2019) advises a pH range of 6.5 to 8.0 and does not recommend the use of buffers but states that the pH of the stock solution should be adjusted within the range of 6.0 to 8.5 to have the more toxic form of the test chemical.

TABLE 2: pH tolerance for species used in selected representative acute aquatic toxicity testing and specifications

| Biological species | pH tolerance range | Specifications/recommendations | Recommended buffer | Target range | Guideline(s) |
|--------------------|--------------------|--------------------------------|--------------------|--------------|--------------|
| Green algae        | 6.4–9.6            | CO2                            | Within 0.5 pH units | OECD TG 201  |
| Daphnia magna      | 6–9                | None                           | No drift beyond 6–9 | OECD TG 202  |
| Fish               | 6.0–8.5            | No buffer to be used, HCl and NaOH | Adjust stock solution to pH 6–8.5 | OECD TG 203  |
| Zebrafish embryo   | 6.5–8.5 (can tolerate 5–10) | No recommendation in the guideline | None | Within 1.5 pH units | OECD TG 236  |

OECD TG = Organisation for Economic Co-operation and Development test guideline.
Our analysis of literature data for the acute fish embryo toxicity test with the zebrafish (Danio rerio) revealed that no information on the pH of the test medium during or after exposure was provided for 71% of 83 studies (Klüver et al. 2019). Often, only the pH of the medium itself was adjusted but not buffered. Only in 10% of the studies was adjustment of the pH specifically mentioned, and in 19% the reported pH ranged over several units (e.g., 6.5–7.8; Klüver et al. 2019). Even if the studies were conducted according to OECD test guideline 2013, pH control would not be assured because the guideline is based on an unbuffered International Organization for Standardization (ISO) water as test medium and requires that the pH does not vary by more than 1.5 pH units in the range of 6.5 to 8.5 (Organisation for Economic Co-operation and Development 2013). Zebrafish embryos can tolerate pH values between 5 and 10 (Andrade et al. 2017), so a large shift in pH may go unnoticed in controls but may affect IOC speciation and resulting effects in test treatments. Bittner et al. (2018) demonstrated that Good’s buffers are suitable to adjust the pH within the range that is tolerable for zebrafish embryos.

Hence, even if bioassays are performed according to OECD guidelines, additional measures should be taken to adjust and buffer the pH. Table 3 summarizes the buffers that were evaluated and recommended by Rendal et al. (2012) and Bittner et al. (2018). The buffer strength should be high enough such that no shift in pH occurs during the experiment. Often, a range of 5 to 10 mM (Rendal et al. 2012) is acceptable, but it can be as high as 40 mM (Bittner et al. 2018), provided that there are no negative effects of the buffer on control performance. Rendal et al. (2012) were able to perform algal toxicity and Daphnia toxicity assays with minimum pH drift using the recommended buffers at concentrations of 2 mM and higher but excluded certain buffers such as phosphate, N-cyclohexyl-2-amino propane sulfonic acid, and N-cyclohexyl-3-amino propane sulfonic acid because of toxicity and pH drift. Bittner et al. (2019b), in contrast, reported that buffers in concentrations up to 40 mM had to be applied to keep the pH constant in zebrafish embryo toxicity experiments (Table 3).

In summary, performing bioassays according to approved OECD or ISO test guidelines cannot assure that reliable toxicity estimates can be obtained for IOCs. The buffers recommended by Rendal et al. (2012) and Bittner et al. (2018) are the first choice in aquatic toxicity testing, but control experiments are necessary in all cases to assure pH stability and negligible toxicity of the buffered test medium. Our present experience is mainly related to acute toxicity testing. If IOCs are tested in chronic toxicity studies, even greater care must be taken to assure that the pH remains constant during the experiment and that the buffers used are nontoxic for the entire duration of the chronic toxicity experiment.

**Experimental ecotoxicity studies**

In 2011, a critical review article on the pH dependence of bioaccumulation and toxicity of IOCs was published...
(Rendal et al. 2011b). The reader is referred to this excellent article and the papers cited therein, which cover algal, *Daphnia*, and fish toxicity. Since publication of this review, a number of additional studies have allowed expansion of this earlier data set. Almost all of the newer studies relied on buffers to keep the pH constant, and some measured test exposure concentrations. We revisited some of the earlier studies and updated the 2011 literature review (Web of Science search with [acid or base or ion* or speciation] and organic and toxicity and pH and [algae or daphn* or fish] and 2011–2019; last update 8 September 2019). Experimental data from the literature are compiled in Supplemental Data, Table S1, together with corresponding physicochemical properties that are used in this corresponding review article to evaluate different ecotoxicity prediction methods.

Additional algal toxicity work included in Supplemental Data, Table S1, analyzed the pH dependence of basic pharmaceuticals (Neuwoehner et al. 2011), 6 acidic and basic pharmaceuticals at 3 pH values (Rendal et al. 2012). Not included in Supplemental Data, Table S1, was the review on the algal toxicity of triclosan, with new measurements at pH 7, 8 and 8.5, that showed similar 10% effect concentration (EC10) values for biomass and growth rate at pH 7 and 8 but 10 times lower toxicity at pH 8.5 (Roberts et al. 2014).

Plants were not included in Supplemental Data, Table S1, for the low number of literature studies identified. Phytotoxicity of carboxylic acid was tested by Himanen et al. (2012) at pH 6. The toxicity of 4 sulfonyleurea herbicides toward *Lemma gibba* was found to be pH-dependent, showing decreasing toxicity with increasing pH (Rosenkrantz et al. 2013).

The database for invertebrates from Rendal et al. (2011b) was expanded by a study of chloroquine in *Daphnia* (Rendal et al. 2011a), 6 acidic and basic pharmaceuticals in *D. magna* (Boström and Berglund 2015), and the antibiotic sulfadiazine in *D. magna* (Chen and Lin 2016). The studies from Boström and Berglund (2015) as well as the previously compiled studies from Zhao et al. (1998), Cronin et al. (2000), and Kamaya et al. (2005a, 2005b) are also included in Supplemental Data, Table S1.

Toxicity data of phenolic compounds (Pirselova et al. 1996) and carboxylic acids (Seward and Schultz 1999) using *Tetrahymena pyriformis* were also included in our updated database. Earlier work on the pH dependence of IOC toxicity to fish included studies of phenols and carboxylic acids on fathead minnow (Saarikoski and Vilukela 1982; Holcombe et al. 1984), fatty acids in various fish species (Onitsuka et al. 1989), substituted phenols in carp (Kishino and Kobayashi 1995), and a diverse data set on fish toxicity compiled by Kipka and Di Toro (2009). The pH-dependent acute fish toxicity of sertraline was reported by Valenti et al. (2009). The zebrafish embryo assay was applied to various β-blockers (Bittner et al. 2018), anti-histamines (Bittner et al. 2019b), and acidic pharmaceuticals (Bittner et al. 2019a). Another recent study on pharmaceuticals in the zebrafish embryo assay showed higher toxicity with higher fraction of neutral species (Alsop and Wilson 2019). We did not include a study on the pH-dependent toxicity of glyphosate toward zebrafish embryos in Supplemental Data, Table S1, because the zebrafish embryo assay was performed unbuffered and pH was <4, most likely leading to pH effects rather than effects of glyphosate because glyphosate did not cause any mortality up to 10 mM when buffered to pH 7 and the negative control was almost equally toxic at low pH values (Schweizer et al. 2019).

Triclosan (pKₐ 8.1) is probably the most thoroughly investigated IOC with regard to pH-dependent toxicity across diverse aquatic organisms (Franz et al. 2008; Roberts et al. 2014; Khattak et al. 2018; Li et al. 2018). These studies show increased toxicity with decreasing pH, but because of the photolability of the anionic species (Tixier et al. 2002), some earlier studies, especially on algal toxicity conducted without UV filters, have to be treated with caution. We therefore excluded triclosan in this analysis. We also did not attempt to compile the vast toxicity literature on bacteria and yeast because this was outside the scope of the present effort.

**BIOCONCENTRATION**

Experimental bioaccumulation of IOCs and associated prediction models have been recently reviewed (Armitage et al. 2017). Models range from simple equilibrium partitioning models to time-resolved toxicokinetic models. Physiologically based fish bioaccumulation models describe the uptake via gills for acids (Erickson et al. 2006a, 2006b) and bases (Nichols et al. 2015).

Experimentally, the bioconcentration factor (BCF) is defined by the ratio of the uptake clearance and elimination rate constants, $k_{uptake}$ and $k_{elimination}$, or the ratio of internal concentration, $C_{internal}$, to external concentration, $C_{external}$, at steady state (Equation 4).

$$\text{BCF} = \frac{k_{uptake}}{k_{elimination}} \rightarrow \text{steady state} \rightarrow \text{BCF} = \frac{C_{internal}}{C_{external}} \quad (4)$$

It has been recommended that $K_{OW}$-based models developed for neutral chemicals could be adapted to IOCs simply by replacing the $K_{OW}$ by the ionization-corrected $D_{OW}(pH)$ (Fu et al. 2009), assuming that the charged species plays no role in uptake because $D_{OW}(pH) = D_{neutral} \cdot K_{OW}$ (Schreiber et al. 2011). This assumption is justified for octanol, where organic ions distribute only as ion pairs or with a counterion (Johnson and Westall 1990; Escher and Schwarzenbach 1996; Chen and Lin 2016). However, in the case of IOCs, octanol is not a suitable surrogate for biological matrices. In fact, organic ions have quite a strong affinity to membrane lipids even if exhibiting limited partitioning into storage lipids (Escher and Sigg 2004), and anions often have a higher affinity to proteins than their neutral counterparts (Henneberger et al. 2016a, 2016b).

Simple mass-balance models have been invoked to predict the steady-state concentrations in aquatic organisms (Bittermann et al. 2018; Goss et al. 2018). The main limitation of a mass-balance approach is that an aquatic organism is not necessarily in equilibrium with the surrounding water. A steady state is more likely to be reached for small aquatic organisms such as algae,
Daphnia, and fish embryos during standard exposure durations of acute toxicity tests. Further, achievement of steady state is also heavily influenced by metabolism demonstrated by work on fish embryos (Brox et al. 2014, 2016a, 2016b), daphnids (Kretschmann et al. 2011), and gammarids (Kretschmann et al. 2012) for neutral chemicals; but it is also a relevant consideration for IOCs.

Models that describe the pH dependence of bio-concentration of IOCs often assume that the neutral species can be taken up faster into aquatic organisms. Trapp and Horobin (2005) developed an ion-trapping model for the uptake of neutral and corresponding charged chemicals into generic tumor cells and their mitochondria. Zarfi et al. (2008) developed a mechanistic model for the uptake of sulfonamides in bacteria that relies on reduced diffusion coefficients of the charged species across membranes. Fu et al. (2009) published a literature review and derived a similar electrostatic model for the uptake into cells.

Ion-trapping models have to be considered if there is a pH difference between external and internal aqueous phases (cytosol, hemolymph, blood, etc.). Organisms maintain a constant internal pH (and often organ- and tissue-specific pH values). The literature is scarce on the internal pH in aquatic organisms (Table 4), and there might be some variability between different compartments and between species; the measurements are difficult and prone to many uncertainties. The ion-trapping model is not necessary if the test pH is close to the internal pH of the organism (Table 4) and no explicit pH dependence of uptake and toxicity is measured. In all other cases a full (no permeability of the ion) or kinetic (smaller permeability of the ion than of the neutral species) ion-trapping model may improve the interpretation and prediction of pH-dependent toxicity. Bittner et al. (2019a) compared the simple mass-balance model with the 2 ion-trapping models coupled to an internal mass-balance model to describe the apparent BCF in zebrafish embryos measured after 96 h of exposure. All 3 models yielded predictions generally within a factor of 10 to the experimental BCF, but the ion-trapping models described the pH dependence better than the mass-balance model (Bittner et al. 2019a).

**MODES OF ACTION OF IOCs**

Figure 2 presents a flowchart that allows the assignment of the 3 main types of toxicity mechanisms for IOCs (baseline toxicity, uncoupling of oxidative phosphorylation, and receptor binding) and associated predictive models. As a first step, the physicochemical properties and information on the mode of action need to be collected from the literature. The first decision point is related to the speciation. Multiple species are only relevant for acids with $pK_a < 9$ and bases with $pK_b > 5$. Next, one must decide if toxicity can be directly predicted or if the toxicokinetics of the IOC first needs to be considered. The latter decision applies if the external pH, at which the bioassay was conducted, is more than one unit from the internal pH of the biological species tested (decision criterion: $pH_{\text{external}} \geq pH_{\text{internal}} \pm 1$). In this case, a toxicokinetic model needs to be applied to account for the ion-trapping effect (see section Bioconcentration), and this model can be integrated directly into ecotoxicity prediction models, as outlined in the section Ion-trapping model to explain the pH dependence of toxicity.

The next decision in the framework questions if the target site is membrane lipids or proteins/receptors (Figure 2). For those IOCs that intercalate into membranes, one can differentiate IOCs that act as baseline toxicants and those that are uncouplers.

Baseline toxicants, compounds that only act via their minimal toxicity by narcosis, trigger effects via disturbance of membrane structure and functioning at constant membrane concentrations (McCarty and Mackay 1993; van Wezel and Opperhuizen 1995; Figure 2). Prediction models include QSARs for baseline toxicity (see section Baseline toxicity [narcosis]) and the concept of critical body burden (see section Critical body burden concept), as formalized in the target lipid model (see section Target lipid model for baseline toxicity).

If the IOC is a phenol or contains an acidic amine function (i.e., N-acidic) and its target site is the membrane, specifically the mitochondrial membrane, it can act as an uncoupler of oxidative phosphorylation (Figure 2; prediction models are discussed in more detail in the section Uncouplers). The mechanism underlying uncoupling is a protonophoric shuttle mechanism, where the neutral species carries and releases protons across the mitochondrial membrane, destroying the electrochemical proton gradient that is needed to drive adenosine triphosphate synthesis (Terada 1990). The toxicity of uncouplers is highly pH-dependent (i.e., can vary by a factor of 20 within pH of 5–9 [Escher et al. 1999]), with a maximum effect occurring when the internal pH of the organism is close to the $pK_a$. At higher pH values, an additional uncoupling effect can be driven by a heterodimer formed between the neutral and anionic phenol species (Escher et al. 1999). The pH dependence on toxicity is thus bimodal, accounting for the monomeric and the heterodimeric shuttle mechanisms against a backdrop of baseline toxicity, as depicted in Figure 2 (adapted from...
Predictive models for estimating un-coupling will be presented below (see section Uncouplers).

If the target is a protein (e.g., a nuclear receptor or an enzyme), be it located in the membrane or in the cytosol, in the nucleus, or at any interface, one can invoke binding models that account for different binding affinities of each IOC species (Figure 2). Binding affinity models may originate from 3-dimensional structures of characterized targets or be built as numerical models trained on the activity data from the Chembl or Pubchem activity data or the ToxCast/Tox21 assays, as is the case for the pocketome model described below (see section Binding to proteins and receptors).

The extended fish plasma model (FPM) is a special case of protein binding models (Figure 2). It applies to pharmaceuticals and assumes that toxicodynamics in fish are similar to those in humans such that plasma concentrations in fish that are equal to human therapeutic plasma concentrations pose a potential risk to the fish health. At present, the FPM is based on \( K_{OW} \) (Huggett et al. 2003) or octanol–water distribution ratio (\( D_{OW}[pH] \)) for IOCs (Schreiber et al. 2011); but as indicated in the flowchart, full toxicokinetics and pH dependence of protein binding and receptor binding of IOCs need to be accounted for, as described below (see section The fish plasma model for IOCs).

ECOTOXICITY PREDICTION MODELS

**Mixture toxicity of the different species**

Early work has interpreted the pH dependence of toxicity, expressed as \( EC_{w}(pH_{external}) \), as only attributable to the effect of the neutral species \( (EC_{neutral}) \) weighted by the fraction \( \alpha_{neutral} \) of the neutral molecule (Equation 5).

\[
EC_{w}(pH_{external}) = EC_{neutral} \cdot \alpha_{neutral}
\]  

This model assumes that only the neutral species are bioavailable and has been shown to perform poorly for describing the pH-dependent effects of monoprotic acids and bases to D. magna (Boström and Berglund 2015). This model is unrealistic because it would predict no toxicity for fully charged chemicals. This cannot be the case because even chemicals that are permanently charged are taken up by organisms and cause adverse effects.

An alternative model construct is to assume that both neutral and charged species of an IOC may contribute to the overall effect, either with the same intrinsic potency (baseline
toxicity) or with different potencies; for example, a neutral species may have a higher or a lower affinity to a receptor than a charged species. The simplest way of modeling such a combined effect is a concentration addition (CA) mixture toxicity model (Altenburger et al. 2000). Concentration addition applies to chemicals with the same mode of action, and we can safely assume that 2 different species of the same molecule have the same mode of action (with the exception of uncoupling, which is separately discussed in the section Uncouplers). The effect concentration of concentration addition, EC_{CA}, can be calculated by Equation 6 (Altenburger et al. 2000), where EC(i) is the effect concentration (e.g., median lethal concentration [LC50] or EC50 or EC10) of the individual mixture components and \( \alpha_i \) is the fraction of mixture component \( i \)—in the case of IOCs, of species \( i \).

\[
EC_{CA} = \left( \sum_{i=1}^{n} \alpha_i \right)^{-1} \tag{6}
\]

Equation 6 can be rearranged for organic acids to Equation 7, where the 2 species \( i \) are the neutral acid form (HA, \( \alpha_{HA} \)) and the charged anionic species (\( A^- \), \( \alpha_{A} \)) and is described by an analogous equation for bases (Neuwoehner and Escher 2011).

\[
\frac{1}{EC} = \frac{\alpha_{HA}}{EC(HA)} + \frac{\alpha_{A}}{EC(A^-)} \tag{7}
\]

The effect concentration of both species, EC(HA) and EC(A^-), can be derived from the slope and intercept of a linear regression of the inverse of the measured effect concentration of the mixture (1/EC) against the fraction of neutral species (Figure 3). If the experimental EC equals EC_{CA} or, in other words, if Equation 7 delivers a good linear regression, then concentration addition is confirmed. Because this analysis relies on the external EC, the differences in EC(HA) and EC(A^-) could be attributed to toxicokinetic or toxicodynamic differences of these species.

This mixture model was used to explain the pH-dependent effects of many chemicals toward Alivibrio fischeri (Baumer et al. 2017), but its disadvantage is that the fit can only be good if the species fraction spans a wide range (i.e., 1–99%). This is not always the case in the experimentally accessible pH range, so application of Equation 7 for analysis of pH-dependent toxicity is often limited. Further, this mixture model does not provide any mechanistic information but can at least give an indication if the different species of an IOC are acting in a concentration addition manner.

**Baseline toxicity (narcosis)**

Narcosis or baseline toxicity is the minimum toxicity of any chemical caused by intercalation into biological membranes (McCarty and Mackay 1993; van Wezel and Oppenhuizen 1995). For neutral organic chemicals acting as baseline toxicants, simple QSARs can be used to predict the toxicity from physicochemical descriptors of hydrophobicity, such as \( K_{OW} \) (Mackay et al. 2009).

If toxicity is determined at a pH value that is <1 log unit from the internal pH of cells and organisms, then we can apply existing QSAR models for the analysis of baseline toxicity (Figure 4A) simply by replacing the typical descriptor for hydrophobicity, log \( K_{OW} \), by the ionization-corrected biomembrane–water distribution ratio, \( D_{lip/w}(pH) \). The membrane is typically simulated by liposome, membrane bilayer vesicles so that the liposome–water distribution ratio \( D_{lip/w}(pH) \) serves as the surrogate parameter partitioning into biomembranes (Escher and Schwarzenbach 2002).

The \( D_{lip/w}(pH) \) (Equation 8) is composed of the liposome–water partition constant \( K_{lip/w}(i) \) of the species \( i \) and the fraction of species \( \alpha_i \) (Equations 1 and 2).

\[
D_{lip/w}(pH) = \sum_{i=1}^{n} \alpha_i \times K_{lip/w}(i) \tag{8}
\]

These types of baseline toxicity QSAR models have been adapted for IOCs in acute toxicity tests with the bioluminescence bacterium Alivibrio fischeri (Escher et al. 2017), green algae (Escher et al. 2006; Neuwoehner et al. 2008), D. magna (Escher et al. 2006), fish embryos (Klöver et al. 2019), and guppy fish (Escher and Schwarzenbach 2002), as summarized in Table 5 (Equations 9–14). The use of the ionization-corrected \( D_{lip/w}(pH) \) is an implicit application of the mixture toxicity model of concentration addition, assuming that all species have the same potency once inside the membrane.

A baseline toxicity QSAR may not only serve to identify if an IOC acts as a baseline toxicant but can also quantify the magnitude of a specific toxic, which is typically expressed as the toxic ratio (TR; Equation 15; Verhaar et al. 1992).

\[
TR = \frac{LC50 (baseline\ toxicity)}{LC50 (experimental)} \tag{15}
\]

**Critical body burden concept**

The critical body burden (also called “critical body residue”) hypothesis (McCarty and Mackay 1993), which asserts that internal tissue concentrations provide a better dose metric for describing toxicological responses than external media concentrations, provides a general paradigm that is increasingly used in aquatic hazard assessment (Meador et al. 2008, 2011; McCarty et al. 2011).
The concept of constant membrane concentrations of baseline toxicants was initially derived for nonpolar neutral compounds but expanded to polar compounds (Vaes et al. 1998b; Escher and Hermens 2002) and IOCs (Escher and Schwarzenbach 2002; Escher and Hermens 2004). These critical membrane lipid concentrations (internal LC50 \(\text{ILC50}_{\text{baseline/tissue}}\)) are approximately 200 mmol/kg\text{lipid} independent of the chemical or species form. Direct comparisons of the membrane permeability induced by neutral compounds and IOCs also occurred at the same critical membrane concentrations (Escher et al. 2002).

These critical membrane concentrations also serve as an anchor for the evaluation of specific effects (Figure 4B). Ionizable organic chemicals are considered to elicit a specific effect if they have lower membrane ILC50s than those that trigger baseline toxicity. What makes the concept more difficult to rationalize is that the specific effect is often exerted at a different target site (e.g., in the cytoplasm or the nucleus); hence, toxic ratio analyses are better done on the whole-body burden ILC50 rather than on the membrane-level ILC50\text{membrane} (Escher and Hermens 2002).

For IOCs, all species are expected to act together in a concentration addition manner inside the membrane. Unfortunately, the database is far too small on ILC50s (Meador et al. 2011) and almost nonexistent for IOCs. But that does not mean that the concept does not apply, and it clearly provides benefits for the interpretation of modes of action (Escher et al. 2011a). In its simplest form, a lipid-normalization is done to derive the membrane concentrations from measured total internal concentrations; but that is too simple for IOCs that also bind substantially to proteins or stay partially in the aqueous phase. If we use experimental BCFs or apply the bioconcentration models to derive the BCF from a full mass balance for IOCs in combination with measured ECs triggering the effect \(y\), EC\(y\), then we can derive the ILC50 (or internal effect concentrations \(\text{IEC}y\)) also for IOCs (Equation 16).

\[
\text{ILC50}(\text{pH}) = \text{LC50}(\text{pH}) \times \text{BCF}(\text{pH})
\]

\[\text{(16)}\]

**Target lipid model for baseline toxicity**

The target lipid model (TLM) provides a model framework that is based on the concept of critical membrane concentrations and baseline toxicity and has been applied to describe the acute aquatic toxicity of a wide range of nonpolar and polar nonionic chemicals across test organisms and endpoints (Kipka and Di Toro 2009). This critical body burden model can be applied to large sets of chemicals with only physicochemical properties and measured ECs as input parameters. In the following analysis we build on prior work (Redman et al. 2018) to show that the TLM may also be applied to IOCs but only if there is less than a pH unit gradient between the external medium and the inside of the organisms. If this is not the case, the ion-trapping model for baseline toxicity derived below (see section ion-trapping model to explain the pH dependence of toxicity) should be used.

The biological species-specific critical target lipid body burden (CTLBB, millimoles per kilogram of lipid membrane) can be derived from the measured acute toxicity of chemicals acting by baseline toxicity using Equation 17.

\[
\log(\text{CTLBB}) = \log\left(\text{L(E)}\text{C50}\right) + \log K_{\text{lip/w}}
\]

\[\text{(17)}\]

The \(\text{L(E)}\text{C50}\) (millimolar) is the lethal or effective concentration causing a 50% population response and \(K_{\text{lip/w}}\) is the liposome–water partition constant for the chemical. Acute CTLBBs have been developed for approximately 80 test organisms and include a number of chemical classes including halogen, hydroxyl, ether, and ketone groups and monoaromatic and polyaromatic rings (McGrath et al. 2018). Given CTLBBs and class corrections that account for differences in lipid–water partitioning behavior, acute toxicity for various test organisms can be predicted for any baseline toxicant.

Prior application of the TLM to IOCs (Redman et al. 2018) assumed that neutral and charged species are equipotent in contributing to baseline toxicity and therefore act in a concentration addition manner, as described by Equation 7. For IOCs, Equation 18 is converted from Equation 17 simply by replacing \(K_{\text{lip/w}}\) of the neutral species by \(D_{\text{lip/w}}(\text{pH})\).

\[
\log(\text{CTLBB}) = \log\left(\text{L(E)}\text{C50}\right) + \log D_{\text{lip/w}}(\text{pH})
\]

\[\text{(18)}\]

For monoprotic acids and bases, the \(D_{\text{lip/w}}(\text{pH})\) can be derived from the fraction of neutral species and the \(K_{\text{lip/w}}(\text{neutral species})\) and the \(K_{\text{lip/w}}(\text{charged species})\) according to Equation 19, which

\[
\log(\text{CTLBB}) = \log\left(\text{L(E)}\text{C50}\right) + \log K_{\text{lip/w}}(\text{neutral species}) + \log D_{\text{lip/w}}(\text{pH})
\]

\[\text{(19)}\]
is a simplified version of Equation 8 for one neutral and one charged species.

\[
D_{lip/w}(pH) = \alpha_{neutral} \times K_{lip/w}^{\text{(neutral species)}} + (1 - \alpha_{neutral}) \times K_{lip/w}^{\text{(charged species)}}
\]  

(19)

The \( K_{lip/w}^{\text{(neutral species)}} \) can be well predicted with poly-parameter linear free energy relations (pp-LFER) in the case of neutral compounds (Vaes et al. 1998a; Endo et al. 2011). In the present study, we used experimental \( K_{lip/w}^{\text{(neutral species)}} \) if available and filled data gaps with predictions obtained from the pp-LFER by Endo et al. (2011; Supplemental Data, Table S1).

For the prediction of the partitioning of ions and more complex structures (like zwitterions, multivalent or multifunctional ions), a more mechanistic model is desirable, like COSMOmic (Bittermann et al. 2014, 2016), which relies on fluid-phase thermodynamics and quantum mechanical calculations (Klamt et al. 2008). If \( K_{lip/w}^{\text{(charged species)}} \) was previously estimated with COSMOmic or if experimental values of \( K_{lip/w}^{\text{(charged species)}} \) were available, these values were used. For all other IOCs we used a constant ratio of neutral to charged species, in the log form called \( \Delta mw \) (Equation 20), derived from the experimental data in Supplemental Data, Table S1.

\[
\Delta mw = \log K_{lip/w}^{\text{(neutral species)}} - \log K_{lip/w}^{\text{(charged species)}}
\]  

(20)

Experimental \( \Delta mw \) typically varied approximately between 1 for phenols (Escher and Schwarzenbach 1996), 2 for carboxylic acids (Escher and Sigg 2004), and 0 for N-acidic compounds like benzimidazoles and hydrazones (Spycher et al. 2008b). As demonstrated for 56 anions and 36 cations, the assumption of \( \Delta mw = 1 \) predicted the membrane–water partition constants for anions (\( R^2 = 0.61 \), root mean square error [RMSE] = 0.79) better than for cations (\( R^2 = 0.23 \), RMSE = 1.14; Bittermann et al. 2016). We derived \( \Delta mw \) empirically from the average of the \( \Delta mw \) values that were calculated from experimental partition constants (Supplemental Data, Table S1): \( \Delta mw \) was \( 1.24 \pm 0.51 \) for the acids and \( 0.97 \pm 0.39 \) for the bases.

Inserting Equation 20 into 19 yields Equation 21:

\[
D_{lip/w}(pH) = K_{lip/w}^{\text{(neutral species)}}[\alpha_{neutral} + 10^{-\Delta mw}(1 - \alpha_{neutral})]
\]  

(21)

Equation 21 can be integrated into Equation 18 to provide a simple model framework for predicting the acute toxicity of IOCs (Equation 22).

\[
\log L(E)/C50 = \log(CTLBB) - \log K_{lip/w}^{\text{(neutral species)}}[\alpha_{neutral} + 10^{-\Delta mw}(1 - \alpha_{neutral})]
\]  

(22)

The toxicity data set of Supplemental Data, Table S1, was used to test the TLM framework, excluding a priori those data where uncoupling is the known mode of action and \( pH_{\text{external}} \) outside \( \pm 1 \) the range of \( pH_{\text{internal}} \).
As demonstrated in Figure 5, the log LC50s were generally log-linearly correlated with log $D_{lip,w}(pH)$. Species-specific CTLBBs could be derived from the intercept with the y-axis using a linear regression (Equation 18) in Figure 5. The CTLBB values were not distinguishable between acids and bases; hence, both were regressed together in Figure 5. The CTLBBs were 4 mmol/kglip for green algae (95% CI 2–11, $n=37$, RMSE $=1.155$; Figure 5A), 13 mmol/kglip for crustacea (95% CI 9–19, $n=91$, RMSE $=0.833$; Figure 5B), 17.7 mmol/kglip, for fish (95% CI 15–21, $n=301$, RMSE $=0.704$; Figure 5C), and 37 mmol/kglip, for Tetrahymena (95% CI 28–49, $n=131$, RMSE $=0.721$; Figure 5D). Individual CTLBBs were also calculated for each individual chemical (Supplemental Data, Table S1), and a summary of the range of individual CTLBBs is shown in the Supplemental Data. The CTLBB analysis demonstrates that IOCs that are not assigned as specifically acting gave consistent results with other TLM applications for polar and nonpolar chemicals (Kipka and Di Toro 2009), although the IOCs investigated in the present study all lay at the lower limits of the species–CTLBB distribution of earlier studies (Kipka and Di Toro 2009; Redman et al. 2018). The lower CTLBBs derived in the present study may be attributable to differences in how $K_{lipw}(\text{neutral})$ was estimated in earlier work.

The TLM can be applied across substances to provide a screening estimate of acute toxicity when the bioassay was conducted at approximately pH 7, assuming that the underlying mode of action is baseline toxicity. Further, such models may be particularly useful when compared to empirical toxicity data for identifying potential outlier compounds, thus providing diagnostic insights of a more specific mode of action. The outlier data were generally nitro-substituted compounds or pharmaceuticals, which suggests specific modes of action for these classes of compounds (Figure 5; Supplemental Data, Table S1).

This simple TLM was only applied for pH in the range of pH$_{\text{internal}} \pm 1$ and failed to adequately explain the order of magnitude variation in toxicity that can be observed for individual compounds tested over a wider pH range. Thus, more detailed models are needed to explain the pH-dependent toxicity of individual IOCs, as discussed in the next section.

**Ion-trapping model to explain the pH dependence of toxicity**

The ion-trapping model for toxicity follows directly from the ion-trapping model for bioconcentration. If the $\text{BCF}(pH)$ is known in units of kilograms of organism per liter of water, then the ILC50 can be calculated from the LC50 (mol/L) with Equation 16. Subsequent application of an internal mass-balance model can be used to calculate the ILC50 of the target lipid model (Equation 18).

$$\text{ILC50}_{\text{w}}(\text{pH}) = \frac{1 + 10^{pH_{\text{external}}/pK_a}}{1 + 10^{pH_{\text{internal}}/pK_a}} \times \text{LC50}_{\text{w}} \times \text{IC}_{\text{bio}}$$

(23)

For bases, the ILC50$_{\text{w}}$ can be calculated with a similar model:

$$\text{ILC50}_{\text{w}}(\text{pH}) = \frac{1 + 10^{-pK_a/pH_{\text{internal}}}}{1 + 10^{-pK_a/pH_{\text{external}}}} \times \text{LC50}_{\text{w}} \times \text{IC}_{\text{bio}}$$

(24)

If both species can be taken up but the uptake of the neutral species is faster than that of the charged species, we can apply the kinetic ion-trapping model (Equation 25), which was based on earlier models for the uptake of ions into human cells.
(Trapp and Horobin 2005) or bacteria (Zarfl et al. 2008) and applied previously to predict IEC50 in zebrafish embryos (Bittner et al. 2019a) in relation to a given pair of external and internal pHs (Table 4). The same equations also hold for EC50 as for LC50.

$$ILC50_w(PH_{int}) = LC50(PH_{ext}) \times \frac{\gamma_{ext,n} \times \alpha_{ext,n} + 10^{-3.5} \times \frac{N}{2^{N-1}} \times \gamma_{int,n} \times \alpha_{int,n}}{\gamma_{ext,n} \times \alpha_{ext,n} + 10^{-3.5} \times \gamma_{int,n} \times \alpha_{int,n} \times \alpha^{h}}$$ (25)

The difference between the uptake of the neutral and the charged species is driven by differences in the activity coefficients γ of the neutral and charged species between the external aqueous phase (γext,n and γion,ext) and internally (γint,n and γion,int) as well as by the ratio of the membrane permeability between neutral and charged species, Pneutral to Pin. This ratio is approximately 1000 to 10 000; we used Pin = 10^{-3.5} \times P_{ex} as in previous studies (Zarfl et al. 2008; Fu et al. 2009). The Nernst-Planck equation with N = zFE/RT was used to describe the motion of the ionic species across the membrane. A more detailed derivation of Equation 25 is given by Bittner et al. (2019a).

The full ion-trapping model helped to explain the pH-dependent toxicity of organic acids and aliphatic bases in green algae (Escher and Hermens 2004; Neuwoehner and Escher 2011) and has also been invoked to qualitatively explain the accumulation of IOCs in green algae (Vogs et al. 2015). The ion-trapping model for algae was based on the observation that the algal intracellular pH is often higher than the external pH of the medium, in which bioassays are conducted (Küsel et al. 1990). Thus, the neutral species, which is readily membrane-permeable, is taken up into the cell and equilibrates between neutral and charged species according to the pH and its acidity constant, leading in case of weak organic bases to a lower IEC50w than EC50 if the internal pH is lower than the external pH (Neuwoehner and Escher 2011) and vice versa for acids (Escher and Hermens 2004). The published ion-trapping model with algae was what we now term the full ion-trapping model (Equations 23 and 24) and considered the internal concentrations in the cytosol, IEC50w,internal, but not the membrane concentrations, the actual target site of baseline toxicants.

We have remodeled the experimental data from these studies (Escher and Hermens 2004; Neuwoehner and Escher 2011) with the kinetic ion-trapping model (Equation 25). There was no large difference in IECw predicted with the full and the kinetic ion-trapping model, so for clarity of presentation only the results of the kinetic ion-trapping model are depicted in Figure 7. As discussed in the section Target lipid model for baseline toxicity, the TLM describes the baseline toxicity sufficiently well if the pH is in the range of pHinternal ± 1; but for larger deviations of the pH, ion trapping becomes effective.

The IEC50w can be interpreted by applying the appropriate baseline toxicity QSAR with D_{lipw(PH_{int})} as the hydrophobicity descriptor. Depending on the pH, some of the organic bases, namely, fluoxetine, norfluoxetine, and propranolol, appeared to have a toxic ratio >10 calculated from external EC50. This apparent toxicity enhancement disappeared when aqueous internal IEC50w values were calculated. Given the almost constant internal pH and little variability in the IEC50w, we plotted the average IEC50w and compared it with the internal baseline toxicity QSAR for IEC50w, which is the same as the EC50 QSAR, only the D_{lipw} at the internal pH was used as the hydrophobicity descriptor. Now, the toxic ratio was pH-independent internally and below 10 (Figure 7). Two of the 3 acids, 2,4-dichlorophenol and 2,4,5-trichlorophenol (Escher and Hermens 2004), were within the range of baseline toxicants for the LC50 and the ILC50w, which can be explained by the fact that their pK_a was very close to the pH of the measurements. In contrast, for 3,4-dinitrophenol with a lower pK_a of 4.2 the ion-trapping model was necessary to bring the ILC50w to baseline toxicity. Note that
3,4-dinitrophenol and 2,4,5-trichlorophenol are weak uncouplers; they will also be discussed in the section Uncouplers.

In a further step, the critical membrane concentration \( \text{ILC}_{50\text{membrane}} \) or \( \text{IEC}_{50\text{membrane}} \) can be approximated by multiplying \( \text{ILC}_{50w} \) or \( \text{IEC}_{50w} \) with \( \frac{D_{\text{lip}}}{w(pH)} \) \( \text{pH}_{\text{internal}} \); Equation 26.

\[
\text{ILC}_{50\text{membrane}} = \frac{D_{\text{lip}}}{w(pH)} \times \text{ILC}_{50w}(\text{pH}_{\text{internal}})
\]

The modeled internal membrane concentrations all fell within the range of baseline toxicity (Figure 7). An analogous ion-trapping model has been applied in plant research to describe the mobility of acidic xenobiotics that comprise the basic phloem sap (Hsu et al. 1996).

We also applied the ion-trapping models to pH-dependent toxicity data for several acidic and basic pharmaceuticals in \( D. \text{magna} \) (Boström and Berglund 2015). Already the ionization-corrected \( D_{\text{lip}}/w(pH) \)-based QSAR model for the nominal concentrations performed quite well, and all ECs fell in the range of baseline toxicity (Figure 8). The internal pH of \( D. \text{magna} \) was not available, so we used the \( \text{pH}_{\text{internal}} \) of 8.44 from \( D. \text{aphnia pulex} \) (Table 4) for the model. The kinetic ion-trapping model gave better results than the full ion-trapping model (data not shown). The bases were well within the range of baseline toxicity without and with the ion-trapping model, but the difference between the different pH values became smaller with the ion-trapping model. The acids were overcorrected by the ion-trapping model and showed apparent specific toxicity for the \( \text{IEC}_{50w} \) and \( \text{IEC}_{50\text{membrane}} \) (Figure 8). This analysis shows the limitations of the ion-trapping model, especially in the case of \( D. \text{magna} \), where the internal pH had to be read across from another \( D. \text{aphnia} \) species.

The toxicity of \( \beta \)-blockers (Bittner et al. 2018) and antihistamines (Bittner et al. 2019b) in the 96-h zebrafish embryo toxicity assay increased (the LC50 decreased) with an increasing fraction of the neutral species. The pH dependence of toxicity of several acidic and basic pharmaceuticals, including
the β-blockers from Bittner et al. (2018) in zebrafish embryo, could also be predicted by both full and kinetic ion-trapping models satisfactorily (Bittner et al. 2019a).

Uncouplers

Uncouplers of oxidation and photophosphorylation have very distinct structural alerts: they are typically acidic IOCs with good membrane permeability of the anionic species, such as substituted phenols or N-acids (Terada 1990). Triclosan acts as an uncoupler, as has been evidenced in isolated mitochondria but also in zebrafish embryos (Shim et al. 2016).

The mechanism of uncoupling and the pH dependence of uncoupling are depicted in Figure 2. It is possible to measure the intrinsic uncoupling activity in isolated energy-transducing membranes (Escher et al. 1997). Baseline toxicity is also accessible in such systems (Escher et al. 2002), and hence toxic ratio can be derived for intrinsic uncoupling (Escher and Schwarzenbach 2002). The pH dependence of the intrinsic uncoupling activity has been described by a kinetic model (Escher et al. 1999), and QSARs exist for the prediction of the intrinsic uncoupling activity that are focused on the rate-limiting step of the uncoupling process, the permeation of the charged species across the membrane (Spycher et al. 2008a, 2008b). The permeability of the lipid bilayer can also be measured in independent experiments or predicted from physicochemical descriptors (Ebert et al. 2018).

The toxic ratio from the in vitro uncoupling assay (TR$_\text{in vitro}$) derived from the measured in vitro activity as well as the QSAR predictions of the in vitro activity can be used to predict the toxicity of uncouplers in aquatic organisms by dividing the LC50$_\text{baseline toxicity}$ from baseline toxicity QSARs of the given aquatic organism by the TR$_\text{in vitro}$ (Equation 27).

$$ LC50_{\text{vivo}} = \frac{LC50_{\text{baseline toxicity in vivo}}}{TR_{\text{in vitro}}} $$

(27)

We binned the TR$_{\text{in vitro}}$ from the literature (Escher and Schwarzenbach 2002) into 3 ranges: TR$_{\text{in vitro}} < 10$ corresponds to baseline toxicity, the range of $10 < TR_{\text{in vitro}} < 100$ was classified as "weak uncoupler," and $100 < TR_{\text{in vitro}} < 1000$ was classified as "strong uncoupler." Then, for a series of phenolic compounds, the baseline toxicity was predicted using published QSARs for ionizable chemicals (Klüver et al. 2019; Escher and Schwarzenbach 2002), and the LC50$_{\text{vivo}}$ was predicted with Equation 27. The overall agreement between experiments and prediction was very good for less potent compounds, many of which were classified as baseline toxicity. The prediction model appeared to have the tendency to overpredict the LC50 of more potent chemicals, as is evidenced by a larger deviation of the higher 1/LC50 values from the 1:1 line in Figure 9, that is, for more potent uncouplers with LC50 < 1 µM.

Binding to proteins and receptors

The IOCs of interest including their metabolites can be studied for their binding activities to proteins characterized by X-ray crystallography, binding assays, or activity assays. A particularly useful approach is to define the “Pocketome” as the ligand binding domain of receptors to simplify the computational needs and to target the specific binding of ligands that trigger biological activity. The Pocketome is based on a comprehensive set of macromolecular binding pockets characterized by X-ray crystallography (An et al. 2005; Abagyan and Kufareva 2009; Kufareva et al. 2012b; Abagyan 2018). This set of pocket models can be used to dock any chemical compound in its neutral and/or charged state at a given pH to all those models and calculate the binding score and/or predict the binding free energy. Depending on the strength of those interactions and the nature of the target, one can predict a likely adverse effect and/or mechanism of action (Kufareva et al. 2012a) and explore the role of speciation for protein binding.

The first set of models of nuclear receptors, a class of protein targets for selected environmental endocrine disruptors, was set and tested by Park et al. (2010). This set included receptors of androgens, estrogens, steroids, and receptors associated with organism development and immune system. The concept was formulated as a possible first step in the prioritization of environmental compounds for testing (Schug et al. 2013). The set of models for predicting the effects of IOCs was extended (Chen et al. 2014) and used successfully to predict the target of the antiparasitic drug praziquantel (Chan et al. 2017). The models were also applied to screen large databases...
of chemicals, for example, estrogenic compounds that included IOCs, for their likelihood to bind to the ligand-binding domain of the estrogen receptor (McRobb et al. 2014).

The charge state of each compound can be generated at different pHs and docked in a relevant charged state. The docking is performed to a multiconformational ensemble of pocket conformations and is further supported by the pharmacodynamic density of target binders derived from their crystallographic structures.

Alternatively, reporter gene assays for nuclear receptors may serve as in vitro proxies for binding to receptors. Hundreds of cell-based reporter gene assays have been systematically tested with thousands of chemicals, many of which are IOCs, in the Tox21 and ToxCast initiatives (Judson et al. 2010; Betts 2013; Huang et al. 2016). All resulting ECs are available on the US Environmental Protection Agency’s Chemistry Dashboard (US Environmental Protection Agency 2019) and have the potential to explore the role of speciation for toxicity, but no such attempts have been made yet. One could argue that the nominal concentrations and speciation in cellular assays that are performed typically at physiological pH 7.4 provide a proxy for the concentrations and speciation inside the cells and inside an organism. Hence, the toxicity of chemicals that cause receptor-mediated toxicity could be predicted by the combination of the discussed toxicokinetic models and in vitro data as proxy of toxicodynamics.

The fish plasma model for IOCs

The FPM follows the same line of reasoning as discussed for cell-based assays. If receptors are conserved and similar in humans and fish, then differences in sensitivity between humans and fish reflect toxicokinetic differences. The FPM was based on the assumption that the same plasma concentration that has a therapeutic effect in humans could activate conserved receptors in fish and hence pose a hazard concern. The plasma concentration in fish at steady state (ss), FPC_{ss}, must then be related to the external exposure concentration. In its simplest form the external exposure concentration is related to the FPC_{ss} via the plasma-water partition constant, $K_{plasma/w}$ (Huggett et al. 2003; Equation 28).

$$FPC_{ss} = \text{exposure concentration} \times K_{plasma/w} \quad (28)$$

Following recommendations by Fu et al. (2009), Schreiber et al. (2011) used the ionization-corrected $D_{\text{plasma/w}}(\text{pH}) = \alpha_{\text{neutral}} \times K_{\text{neutral}}$ to expand the FPM from neutral chemicals to IOCs (Equation 29).

$$\log D_{\text{plasma/w}}(\text{pH}) = 0.75 \times \log D_{\text{neutral}}(\text{pH}) - 1.1 \quad (29)$$

In Equation 29, the binding of the ionic species to plasma is neglected. Protein binding of ionic chemicals, especially of anions, can be higher than lipid partitioning (Henneberger et al. 2016a, 2019); therefore, lipid-based surrogates for plasma will always be limited in case of IOCs. Thus, new models will need to be developed to predict the $D_{\text{plasma/w}}(\text{pH})$. Literature is not abundant for $K_{\text{plasma/w}}$ of organic ions (Nichols et al. 2015; Henneberger et al. 2016a), and we are not aware of any study that has explored the pH dependence of $D_{\text{plasma/w}}(\text{pH})$. This lack of experimental data needs to be overcome before predictive models for $D_{\text{plasma/w}}(\text{pH})$ can be advanced.

In addition, the composition of fish plasma and human plasma differs in lipid and protein content (Escher et al. 2011b), and hence we can expect a bias in $D_{\text{plasma/w}}(\text{pH})$ between humans and fish. This issue needs to be explored in a more systematic manner and integrated into the FPM.

Finally, the FPM assumes that the external aqueous concentration is equal to the internal aqueous concentration in fish. However, this assumption is not always justified, as the iontrapping models have demonstrated. Hence, the FPM would need to become also a 2-step prediction model, as we proposed for ecotoxicity predictions of IOCs. The first step is to translate the $D_{\text{plasma/w}}(\text{pH})$ from humans to fish, and the second step is to back-calculate from internal aqueous to external exposure concentrations using an inverse form of the simple iontrapping models or more complex toxicokinetic models (Nichols et al. 2015).

CONCLUSION

Many of the approaches and principles developed for assessing neutral chemicals can be applied to IOCs if the role of speciation is correctly included in toxicokinetic models. Even simple proxies such as the TLM and the ion-trapping models often give a satisfactory prediction of pH-dependent toxicity and corresponding internal effect concentrations, which are the basis for toxicodynamic analyses.

The limited availability of high-quality data at measured and constant pH values confounds the development of predictive methods for ecotoxicity of IOCs. We encourage improved quality control in future studies with an emphasis on keeping the pH constant throughout the experiment using buffered test media. The pH-dependent effects observed for many IOCs appear to be a result of different uptake kinetics given that the few measured internal ECs were independent of the external pH.

An improved understanding of the internal pH in aquatic biota and the pH-dependent uptake of IOCs together with the concentrations that cause harmful effects will greatly improve future ecological risk assessment of IOCs. For site-specific risk assessments, a further consideration is the need to develop models that account for the spatial and temporal variation of pH that occurs under field conditions.

Although the present review has focused on toxicity assessment of single IOCs, a logical future extension of the models discussed is their application to risk evaluation of chemical mixtures that include IOCs.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4602.

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REFERENCES

Abagyan R. 2018. Drug and target discovery by docking to the pocketome. FEBS Open Bio 8:12–12.

Abagyan R, Kufareva I. 2009. The Flexible pocketome engine for structural chemogenomics. Methods Mol Biol 575:249–279.

Alsop D, Wilson JY. 2019. Waterborne pharmaceutical uptake and toxicity is modified by pH and dissolved organic carbon in zebrafish. Aquat Toxicol 210:11–18.

Altenburger R, Backhaus T, Boedeker W, Faust M, Scholze M, Grimme LH. 2000. Predictability of the toxicity of multiple chemical mixtures to Vibrio fischeri: Mixtures composed of similarly acting chemicals. Environ Toxicol Chem 19:2341–2347.

An JH, Totrov M, Abagyan R. 2005. Pocketome via comprehensive identification and classification of ligand binding envelopes. Mol Cell Proteomics 4:754–761.

Andrade TS, Henriques JF, Almeida AR, Soares AM, Scholz S, Domingues I. 2017. Zebrafish embryo tolerance to environmental stress factors—Concentration-dose response analysis of oxygen limitation, pH, and UV-light irradiation. Environ Toxicol Chem 36:682–690.

Armitage JM, Erickson RJ, Luckenbach T, Ng CA, Prosser RS, Armat J, Schimmer K, Nichols JW. 2017. Assessing the bioaccumulation potential of ionizable organic compounds: Current knowledge and research priorities. Environ Toxicol Chem 36:882–897.

Arp HPH, Brown TN, Berger U, Hale SE. 2017. Ranking REACH registered neutral, ionizable and ionic organic chemicals based on their aquatic persistence and mobility. Environ Sci Process Impacts 20:843–853.

Baumer A, Bittermann K, Klüver N, Escher B. 2017. Baseline toxicity and ion trapping models to describe the pH-dependence of bacterial toxicity of pharmaceuticals. Environ Sci Process Impacts 19:901–916.

Betts KS. 2013. Tox21 to date steps toward modernizing human hazard characterization. Environ Health Perspect 121:A228.

Bittner K, Linden L, Goss K. 2018. Screening tools for the bioconcentration potential of monovalent organic ions in fish. Environ Sci Process Impacts 20:843–853.

Bittner K, Spycher S, Endo S, Pohler L, Huniar U, Goss K-U, Klamt A. 2014. Prediction of phospholipid-water partition coefficients of ionogenic organic chemicals using the mechanistic model CosmoMic. J Phys Chem B 118:14833–14842.

Bittner K, Spycher S, Goss KU. 2016. Comparison of different models predicting the phospholipid-membrane water partition coefficients of charged compounds. Chemosphere 144:382–391.

Bittner L, Klüver N, Henneberger L, Mülhenbrink M, Zarfl C, Escher Bl. 2019a. Combined ion-trapping and mass balance models to describe the pH-dependent uptake and toxicity of acidic and basic pharmaceuticals in zebrafish embryos (Danio rerio). Environ Sci Technol 53:7877–7886.

Bittner L, Teixido E, Keddi I, Escher Bl, Klüver N. 2019b. pH-dependent uptake and sublethal effects of antihistamines in zebrafish (Danio rerio) embryos. Environ Toxicol Chem 38:1012–1022.

Bittner L, Teixido E, Sievert B, Escher Bl, Klüver N. 2018. Influence of pH on the uptake and toxicity of β-blockers in embryos of zebrafish, Danio rerio. Aquat Toxicol 201:129–137.

Boström ML, Berglund O. 2015. Influence of pH-dependent aquatic toxicity of ionizable pharmaceuticals on risk assessments over environmental pH ranges. Water Res 72:154–161.

Brox S, Ritter AP, Kuster E, Reemtsma T. 2014. A quantitative HPLC-MS/MS method for studying internal concentrations and toxicokinetics of 34 polar analytes in zebrafish (Danio rerio) embryos. Anal Bioanal Chem 406:4831–4840.

Brox S, Sievert B, Haase N, Kuster E, Reemtsma T. 2016a. Metabolism of colchicic acid in zebrafish embryos (Danio rerio) as determined by liquid chromatography-high resolution-mass spectrometry. Comp Biochem Physiol C Toxicol Pharmacol 185:20–28.

Chan JD, Cupit PM, Gunaratne GS, McConvy JD, Yang Y, Stoltz K, Webb TR, Dosa P, Roth BL, Abagyan R, Cunningham C, Marchant JS. 2017. The anhelicinal praziquantel is a human serotoninergic G-protein-coupled receptor ligand. Nat Commun 8:1910.

Chen CS, Lin ST. 2016. Prediction of pH effect on the octanol-water partition coefficient of ionizable pharmaceuticals. Ind Eng Chem Res 55:9284–9294.

Chen Y-C, Totrov M, Abagyan R. 2014. Docking to multiple pockets or ligand fields for screening, activity prediction and scaffold hopping. Future Med Chem 6:1741–1755.

Cronin MTD, Zhao YH, Yu RL. 2000. pH-dependence and QSAR analysis of the toxicity of phenols and anilines to Daphnia magna. Environ Toxicol 15:140–148.

Ebert A, Hannesschlaeger C, Goss KU, Pohl P. 2018. Passive permeability of planar lipid bilayers to organic anions. Biophys J 115:1931–1941.

Eddy FB, Lomholt JP, Weber RE, Johansen K. 1977. Blood respiratory dependence and QSAR analysis of the toxicity of phenols and anilines to Daphnia magna. Environ Toxicol 15:140–148.

Endo S, Escher Bl, Goss KU. 2011. Capacities of membrane lipids to accumulate neutral organic chemicals. Environ Sci Technol 45:5912–5921.

Erickson RJ, McMick JM, Lien GJ, Hoffman AD, Battemer SL. 2006a. Uptake and elimination of ionizable organic chemicals at fish gills: I. Model formulation, parameterization, and behavior. Environ Toxicol Chem 25:1512–1521.

Erickson RJ, McMick JM, Lien GJ, Hoffman AD, Battemer SL. 2006b. Uptake and elimination of ionizable organic chemicals at fish gills: II. Observed and predicted effects of pH, alkalinity, and chemical properties. Environ Toxicol Chem 25:1522–1532.

Escher Bl, Ashauer R, Dyer S, Hermens J, Lee J-H, Leslie HA, Mayer P, Meador J, Warne MSJ. 2011a. Crucial role of mechanisms and modes of toxic action for tissue residues of organic chemicals. Int Environ Assess Manag 7:28–49.

Escher Bl, Baumer A, Bittermann K, Henneberger L, König M, Kühnert C, Klüver N. 2017. General baseline toxicity QSAR for non-polar, polar and ionisable chemicals and their mixtures in the bioluminescence inhibition assay with Allivibrio fischeri. Environ Sci Process Impacts 19:414–428.

Escher Bl, Bramaz N, Richter M, Lienert J. 2006. Comparative ecotoxicological hazard assessment of beta-blockers and their human metabolites using a mode-of-action-based test battery and a QSAR approach. Environ Sci Technol 40:7402–7409.

Escher Bl, Cowan-Ellicberry CE, Dyer S, Embry MR, Erhardt S, Halder M, Kwan JH, Johanning K, Oosterwijk MTT, Rutishauser S, Segner H, Nichols J. 2011b. Protein and lipid binding parameters in rainbow trout (Oncorhynchus mykiss) blood and liver fractions to extrapolate from an
that act via the ligand-binding domain of the estrogen receptor α. Toxicol Sci 141:188–197.

Meador JP, Adams WJ, Escher BL, McCarty LS, McElroy AE, Sappington KG. 2011. The tissue residue approach for toxicity assessment: Findings and critical reviews from a Society of Environmental Toxicology and Chemistry Pelsson Workshop. Environ Assess Manag 7:2–6.

Meador JP, McCarty LS, Escher BL, Adams WJ. 2008. 10th anniversary Organisation for Economic Co-operation and Development. 2013. Test No. 236: Fish embryo acute toxicity test. OECD Guidelines for the Testing of Chemicals. Paris, France.

Mölich A, Heisler N. 2005. Determination of pH by microfluorometry: Intracellular and interstitial pH regulation in developing early-stage fish embryos Danio rerio. J Exp Biol 208:4137–4139.

Neuwohner J, Escher BL. 2011. The pH-dependent toxicity of basic pharmaceuticals in the green algae Scenedesmus vacuolatus can be explained with a toxicokinetic ion-trapping model. Aquat Toxicol 101:266–275.

Neuwohner J, Junghans M, Koller M, Escher BL. 2008. QSAR analysis and specific endpoints for classifying the physiological modes of action of biocides in synchronous green algae. Aquat Toxicol 90:8–18.

Nichols JW, Du BW, Berninger JP, Connors KA, Chamblias CK, Erickson RJ, Hoffman AD, Brooks BW. 2015. Observed and modeled effects of pH on bioconcentration of diphenhydramine, a weakly basic pharmaceutical, in fathead minnows. Environ Toxicol Chem 34:1425–1435.

Onitsuka S, Kasai Y, Yoshimura K. 1989. Quantitative structure-toxicity relationship of fatty-acids and the sodium-salts to aquatic organisms. Chemosphere 18:1621–1631.

Organisation for Economic Co-operation and Development. 1984. Test No. 201. Alga, growth inhibition test. OECD Guidelines for the Testing of Chemicals. Paris, France.

Organisation for Economic Co-operation and Development. 2004. Test No. 202. Daphnia sp. Acute immobilisation test. OECD Guidelines for the Testing of Chemicals. Paris, France.

Organisation for Economic Co-operation and Development. 2012. Test No. 211. Daphnia magna reproduction test TG 305. OECD Guidelines for the Testing of Chemicals. Paris, France.

Organisation for Economic Co-operation and Development. 2013. Test No. 236: Fish embryo acute toxicity test. OECD Guidelines for the Testing of Chemicals. Paris, France.

Organisation for Economic Co-operation and Development. 2019. Chemistry Dashboard. [cited 2019 March 22]. Available from: https://comptox.epa.gov/dashboard/

Pisalova K, Balaz S, Schultz TW. 1996. Model-based QSR for ionizable compounds: Toxicity of phenols against Tetrahymena pyriformis. Arch Environ Contam Toxicol 30:170–177.

Redman AD, Parkerton TF, Butler JD, Letinski DJ, Frank RA, Hewitt LM, Bartlett AJ, Gillis PL, Marentette JR, Parrott JL, Hughes SA, Guest R, Bekele A, Zhang K, Morandi G, Wiseman S, Gijsy JP. 2018. Application of the target lipid model and passive samplers to characterize the toxicity of bioavailable organics in oil sands process-affected water. Environ Sci Technol 52:8039–8049.

Rendal C, Kusk KO, Trapp S. 2011a. The effect of pH on the uptake and toxicity of the bivalent weak base chloroquine tested on Salix viminalis and Daphnia magna. Environ Toxicol Chem 30:2395–2406.

Rendal C, Trapp S, Kusk KO. 2012. Critical evaluation and further development of methods for testing ecotoxicity at multiple pH using Daphnia magna and Pseudokirchneriella subcapitata. Environ Toxicol Chem 31:1843–1852.

Roberts J, Price OR, Bettles N, Rendal C, van Egmond R. 2014. Accounting for dissociation and photoysis: A review of the algal toxicity of triclosan. Environ Toxicol Chem 33:2551–2559.

Rosenkranz RT, Cedergreen N, Baun A, Kusk KO. 2013. Influence of pH, light cycle, and temperature on ecotoxicity of four sulfonylurea herbicides towards Lemma gibba. Ecotoxicology 22:33–41.

Saarkoski J, Viluksla M. 1982. Relationship between physicochemical properties of phenols and their toxicity and accumulation in fish. Environ Toxicol Saf 6:501–512.

Schreiber R, Gundel U, Franz S, Kuster A, Rechenberg B, Altenburger R. 2011. Using the fish plasma model for comparative hazard identification for pharmaceuticals in the environment by extrapolation from human therapeutic data. Regul Toxicol Pharmacol 61:261–275.

Schug TT, Abagyan R, Blumberg B, Collins TJ, Crews D, DeFur PL, Dickerson SM, Edwards TM, Gore AC, Guillette LJ, Hayes T, Hendsel J, Moores A, Patitsal HB, Tal TL, Thayer KA, Vandenberg LN, Warner JC, Watson CS, vom Saal FS, Zoeller RT, O’Brien KP, Myers JP. 2013. Designing endocrine disruption out of the next generation of chemicals. Green Chem 15:181–198.

Schwarzenbach RP, Gschwend PM, Imboden DM. 2016. Environmental Organic Chemistry, 3rd ed. John Wiley & Sons, Hoboken, NJ, USA.

Schweitzer M, Brilsauer K, Triebeskorn R, Forchhammer K, Koehler HR. 2019. How photophosphate and its associated acidity affect early development in zebrafish (Danio rerio). PeerJ 7:e7094.

Seward JR, Schultz TW. 1999. QSAR analyses of the toxicity of aliphatic carboxylic acids and salts to Tetrahymena pyriformis. SAR QSAR Environ Res 10:557–567.

Shim J, Weatherly LM, Luc RH, Dorman MT, Neilson A, Ng R, Kim CH, Millard PJ, Gosse JA. 2016. Triclosan is a mitochondrial uncoupler in live zebrafish. J Appl Toxicol 36:1642–1647.

Spycher S, Netzeva TI, Worth A, Escher BL. 2008a. Mode of action-based classification and prediction of activity of uncouplers for the screening of chemical inventories. SAR QSAR Environ Res 19:433–463.

Spycher S, Smejtek P, Netzeva TI, Escher BL. 2008b. Toward a class-independent quantitative structure-activity relationship model for uncouplers of oxidative phosphorylation. Chem Res Toxicol 21:911–927.

Teraha H. 1990. Uncouplers of oxidative phosphorylation. Environ Health Perspect 87:213–218.

Tixer C, Singer HP, Canonica S, Muller SR. 2002. Phototransformation of triclosan in surface waters: A relevant elimination process for this widely used biocide—Laboratory studies, field measurements, and modeling. Environ Sci Technol 36:3482–3489.

Trapp S, Horobin RW. 2005. A predictive model for the selective accumulation of chemicals in tumor cells. Eur Biophys J 34:959–966.

US Environmental Protection Agency. 2019. Chemistry Dashboard. [cited 2019 March 22]. Available from: https://comptox.epa.gov/dashboard/

Vaes WHU, Ramos EU, Verhaar HJM, Cramer CJ, Hermens JLM. 1998a. Understanding and estimating membrane/water partition coefficients: Approaches to derive quantitative structure property relationships. Chem Res Toxicol 11:847–854.

Vaes WHU, Ramos EU, Verhaar HJM, Hermens JLM. 1998b. Acute toxicity of nonpolar versus polar narcosis: Is there a difference? Environ Toxicol Chem 17:1380–1384.

Valenti TW, Perez-Hurtado P, Chamblias CK, Brooks BW. 2009. Aquatic toxicity of sertraline to Pimephales promelas at environmentally relevant surface water pH. Environ Toxicol Chem 28:2685–2694.

vanden Weel AP, Oppenhuizen A. 1995. Narcosis due to environmental pollutants in aquatic organisms: Residue-based toxicity, mechanisms, and membrane burdens. Crit Rev Toxicol 25:255–279.

Verhaar HJM, Van Leeuwen CJ, Hermens JLM. 1992. Classifying environmental pollutants. 1. Structure-activity relationships for prediction of aquatic toxicity. Chemosphere 25:471–491.

Vogt C, Kuehner A, Hug C, Kuester E, Altenburger R. 2015. A toxicokinetic study of specifically acting and reactive organic chemicals for the prediction of internal effect concentrations in Scenedesmus vacuolatus. Environ Toxicol Chem 34:100–112.

Weber AK, Pirow R. 2009. Physiological responses of Daphnia pulex to acid stress. BMC Physiol 9:9.

Zarfl C, Matthis M, Klasmeier J. 2008. A mechanistical model for the uptake of sulfonylureas by bacteria. Chemosphere 70:753–760.

Zhao YH, Ji GD, Cronin MTD, Dearden JC. 1998. QSAR study of the toxicity of benzoic acids to Vibrio fischeri, Daphnia magna and carp. Sci Total Environ 216:205–215.

Zilberstein D, Agramon V, Schuldiner S, Padan E. 1984. Escherichia coli intracellular pH, membrane potential, and cell growth. J Bacteriol 158:246–252.

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