Environmental Toxicology and Chemistry—Volume 41, Number 2—pp. 396–409, 2022
Received: 16 June 2021 | Revised: 21 October 2021 | Accepted: 18 November 2021

Critical Review

Development of Fluoride Protective Values for Aquatic Life Using Empirical Bioavailability Models

Samuel P. Parker,[a,*] Austin E. Wilkes,[b] Gary R. Long,[c] Nigel W. E. Goulding,[d] and Rajat S. Ghosh[e]

[a]EHS Support, Waterbury, Vermont, USA
[b]EHS Support, Hinesburg, Vermont, USA
[c]EHS Support, Collegeville, Pennsylvania, USA
[d]EHS Support, Columbus, Ohio, USA
[e]Alcoa, Pittsburgh, Pennsylvania, USA

Abstract: The derivation of protective values for aquatic life can be enhanced by the development and use of bioavailability models. Recent advances to metals bioavailability modeling are applicable to other analyte groups and should be widely considered. We conducted a meta-analysis of the available aquatic toxicity literature for fluoride to evaluate the utility of hardness, alkalinity, and chloride as toxicity-modifying factors (TMFs) in empirical bioavailability models of freshwater taxa. The resulting optimal multiple linear regression model predicting acute fluoride toxicity to the invertebrate *Hyalella azteca* included all three TMFs (observed vs. predicted 50% lethal concentrations, $R^2 = 0.88$) and the optimal model predicting toxicity to the fish *Oncorhynchus mykiss* included alkalinity and hardness ($R^2 = 0.37$). At >20 mg/L chloride, the preliminary final acute values for fluoride were within 1 order of magnitude and ranged from approximately 18.1 to 56.3 mg/L, depending on water chemistry. Sensitivity of *H. azteca* to low-chloride conditions increased model uncertainty when chloride was <20 mg/L. Because of limited toxicity data, chronic bioavailability models were not developed, and final chronic values were derived using an acute-to-chronic ratio (ACR) approach. Accounting for TMFs, the geometric mean ACR was 5.4 for fish and invertebrate taxa ($n = 6$). The present assessment highlights the need to expand bioavailability modeling to include inorganic anions, particularly fluoride, and demonstrates that existing promulgated protective values for fluoride are likely overly conservative. More toxicological studies are recommended to further refine multivariate empirical bioavailability models for inorganic anions. *Environ Toxicol Chem* 2022;41:396–409. © 2021 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Fluoride; Aquatic; Effect; Bioavailability; Guideline; Benchmark

INTRODUCTION

The derivation of scientifically robust protective values for aquatic life depends on a detailed understanding of the physical and chemical conditions that affect the bioavailability of a substance (Schlekat et al., 2020). Bioavailability is an index of the rate and extent to which a particular substance can reach the active site within an organism (Adams et al., 2020). The extent to which a substance can induce an effect on the active site is influenced by the presence of toxicity-modifying factors (TMFs) in the environment (e.g., water hardness, pH, dissolved organic carbon, alkalinity, and chloride). Substantial advances have been made in understanding and characterizing the role of TMFs using bioavailability models for metals (Adams et al., 2020; Di Toro et al., 2001; Van Genderen et al., 2020; Veltman et al., 2010) that are applicable to other analyte groups. Considering the multitude of studies that have assessed the impact of TMFs on inorganic anions (such as nitrate, sulfate, chloride, and fluoride [Baker et al., 2017; Ji et al., 2020; Mount et al., 2016; Soucek et al., 2011]), the application of similar frameworks may improve our understanding of inorganic anion toxicity to aquatic organisms.

Current frameworks used to quantify bioavailability fall within a continuum of empirical and mechanistic approaches

This article includes online-only Supporting Information.

* Address correspondence to samuel.parker@ehs-support.com
Published online 23 November 2021 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5259

(Brix et al., 2020). Mechanistic frameworks tend to be more sophisticated and require detailed knowledge of effects that occur when a particular compound reacts with a physiologically active site (e.g., biotic ligand model) or established rates of cellular absorption (e.g., mechanistic bioaccumulation models [Di Toro et al., 2001; Veltman et al., 2010]). Empirical frameworks vary in complexity but rely on the ability to predict organismal effects statistically. Trade-offs exist in the development and implementation of these frameworks across the continuum of empirical and mechanistic bioavailability models; however, empirical approaches are often regarded as simpler to implement and more transparent than mechanistic frameworks because they tend to be less heavily parameterized and rely on correlative strength rather than established causal relationships (Brix et al., 2020).

Empirical bioavailability models can leverage univariate or multivariate statistical approaches. The intent of the modeling is to establish predictable and repeatable relationships between TMFs and the effect of a substance on test organisms and receptor groups. This process requires exploratory data analysis, visualizations, and a detailed understanding of statistical assumptions. Univariate approaches, such as simple linear regression, can facilitate rapid assessment of the predictive power of independent variables on effect concentrations. These approaches are widely accepted in the United States and Canada. For example, several promulgated metal protective values use univariate approaches that are based on bioavailability models for surface water hardness, for example, Cd (US Environmental Protection [USEPA], 2016), Cr III (USEPA, 1995), Ni (USEPA, 1995), Ag (USEPA, 1980), and Zn (USEPA, 1995). Univariate empirical approaches have also been developed for inorganic anions. Studies focused on sulfate (Elphick, Davies, et al., 2011; Soucek & Kennedy, 2005), nitrate (Baker et al., 2017; Soucek & Dickinson, 2016), nitrite (Alonso & Camargo, 2008), and chloride (Elphick, Bergh, et al., 2011; Soucek et al., 2011) have identified statistically significant relationships between TMFs and anion toxicity.

Multiple linear regression (MLR) approaches have increased statistical complexity compared to univariate approaches; however, they can provide a greater understanding of the interactions among or between TMFs, which may lead to improved model performance and predictive power. Furthermore, the use of automated model selection and inference tools can greatly increase the efficiency in model optimization when the available toxicological data support large numbers of predictive variables (Calcagno & de Mazancourt, 2010). Multiple linear regression approaches have been widely adopted to assess the bioavailability of metals (Brix et al., 2017; DeForest et al., 2018, 2020; Gillio Meina et al., 2020; USEPA, 2018), and it is recognized that bioavailability models are potentially beneficial to other classes of toxicants as well (Brix et al., 2020). For example, recent research has demonstrated the applicability of bioavailability models for establishing protective values for anionic metalloids (Brix et al., 2001; Ji et al., 2020; Wang & Song, 2021). These studies demonstrate that the bioavailability of anions in freshwater can be mediated by the presence of other water quality parameters and that TMFs are critical to determining robust protective values for inorganic anions.

Several factors are known to moderate or ameliorate the effect concentrations of fluoride, which is a ubiquitous constituent in freshwaters. Divalent metal cations associated with water hardness, such as calcium and magnesium, can form weak complexes with fluoride and have been shown to reduce toxicity in aquatic receptors (Camargo, 2003). Fieser et al. (1986), Pimentel and Bulkley (1983), and Metcalfe-Smith et al. (2003) found that fluoride-forming complexes with polyvalent cations and several other factors can significantly affect the toxicity of fluoride to aquatic organisms. Similarly, Wright (1977) found the presence of calcium to have a pronounced effect on decreasing fluoride toxicity to *Salmo trutta*. There has been limited work focusing specifically on the effect of alkalinity on fluoride toxicity. However, for some metals (e.g., copper), increased alkalinity and the presence of hydroxyl groups were found to form less toxic copper-base complexes (Pagenkopf et al., 1974; Stiff, 1971).

For many taxa, increased chloride concentrations result in decreased fluoride toxicity (Camargo, 2003; Neuhold & Sigler, 1962; Pearcy et al., 2015). The seminal work of Neuhold and Sigler (1962) was among the first on the topic to focus on the ameliorating effect of chloride on fluoride to fish. Neuhold and Sigler (1962) found that acclimating fish (*Oncorhynchus mykiss*) in a chloride-rich environment prior to fluoride exposure increased their tolerance to fluoride. Camargo (2004) found that surface water chloride concentration increased the tolerance of net-spinning caddis to fluoride exposure. Pearcy et al. (2015) conducted acute testing (96 h) on *Hyalella azteca* and *O. mykiss* under varying conditions of water hardness, alkalinity, and chloride to determine the effect of each at modifying fluoride toxicity. The authors concluded that chloride was an important factor in modifying the acute toxicity of fluoride to *H. azteca* and *O. mykiss*. However, Pearcy et al. (2015) did not explore effects among the hardness, alkalinity, and chloride TMFs using MLR or other, more advanced multivariate empirical bioavailability modeling approaches. Understanding the role of other fluoride TMFs using a multivariate approach could help improve estimates of final acute values (FAVs).

Although it is established that TMFs can affect the rate and extent of fluoride effects to aquatic receptors, few FAVs have been derived that incorporate bioavailability models. British Columbia and Quebec, Canada, in addition to Illinois, Michigan, and New York in the United States have FAVs for fluoride that are informed by univariate empirical models. These models are based on the ameliorating effect of hardness on acute fluoride toxicity. Generic acute to chronic ratios (ACRs) were applied in most scenarios to convert hardness-adjusted FAVs to final chronic values (FCVs). Identification and application of ACRs were shown to have a strong influence on the existing FCVs, and a more detailed assessment is needed to understand the role of TMFs on ACRs. Federal guidance in Canada is based on an assessment factor approach that does not consider empirical bioavailability models (Canadian Council of Ministers of the Environment [CCME], 2002). In the United States, promulgated nationally recommended water quality criteria for the protection of aquatic life for fluoride do not exist.

To evaluate the effects of TMFs on fluoride bioavailability, we conducted a meta-analysis on acute ($n = 20$) and chronic ($n = 17$) aquatic fluoride toxicity studies. The TMFs explored included hardness, alkalinity, and chloride. Leveraging the acute toxicological data from Pearcy et al. (2015), empirical bioavailability models were developed for the invertebrate *H. azteca* and the fish *O. mykiss* to evaluate the utility of MLR-based bioavailability models in developing more robust FAVs for fluoride. Those FAVs derived using empirical bioavailability models were compared to FAVs that did not consider TMFs. In addition, the suitability of adopting similar MLR approaches for the derivation of FCVs was explored. Those FCVs that did not consider TMFs were compared to FCVs derived based on ACR-converted FAVs.

MATERIALS AND METHODS

Review of toxicity data

Aquatic toxicity data sets for fluoride were compiled and assessed for suitability in accordance with the USEPA guidelines (Stephan et al., 1985). Toxicity data summarized from the acute ($n = 20$) and chronic ($n = 17$) studies reviewed can be found in Supporting Information, Tables S1 and S2, respectively. Chronic *O. mykiss* tests included from Pearcy et al. (2015) used the test methods described in Lazorchak and Smith (2007). Studies with concurrent measurements of hardness, alkalinity, and chloride were prioritized for review and analysis. Although multiple surface water quality parameters are known to affect fluoride toxicity (e.g., chloride, alkalinity, hardness, pH, and water temperature), subsequent analyses are limited to the influence of hardness, alkalinity, and chloride TMFs because of the availability of suitable data for empirical bioavailability modeling from Pearcy et al. (2015). Toxicity data that did not include TMFs were retained for the purpose of assessing acute and chronic protective values that did not account for TMFs, herein described as non-TMF protective values. Protective values that account for TMFs are not preceded by any modifier text (i.e., FAV denotes that TMFs were considered).

Data analysis

Empirical bioavailability model development was conducted using the acute toxicity data from Pearcy et al. (2015). An MLR modeling similar to the approach described in DeForest et al. (2018) for aluminum was used to evaluate how chloride, hardness, and alkalinity water quality parameters affected acute 50% lethal concentration (LC50) values in the benthic invertebrate *H. azteca* and the fish *O. mykiss*. Acute fluoride LC50 values were assessed separately for *H. azteca* and *O. mykiss*. The MLR models were constructed with LC50 as the response variable and chloride, hardness, and alkalinity concentrations serving as potential predictor variables. The response and predictor variables were natural log–transformed prior to assessment.

Using the R statistical programming language and the glmulti package (Calcagno & de Mazancourt, 2010), every possible combination of explanatory variables was explored to develop candidate models considering main effects for transformed LC50 fluoride concentrations. Within the glmulti package, linear model fitting functions were used for each species instead of generalized linear modeling approaches. Bayesian information criterion (BIC) minimization supported candidate model selection along with quantitative and qualitative metrics of model performance. The ΔBIC was used to provide evidence against one candidate model being selected over another as the best model. The BIC offers a robust approach for confirmatory analysis and variable selection (Aho et al., 2014). It can be advantageous over the Akaike information criterion because of penalties assigned with overfitting. Both adjusted R^2 and predicted R^2 were also calculated for each model, and the potential for multicollinearity was assessed using variance inflation factor (VIF) thresholds.

Consistent with the objectives of the present study, the evaluation focused predominantly on the main effects across the range of water quality conditions tested by Pearcy et al. (2015), which were slightly different for each species. For *H. azteca*, hardness, alkalinity, and chloride ranged from 24 to 306 mg $CaCO_3$/L, from 16 to 108 mg $CaCO_3$/L, and from 4.1 to 95 mg/L, respectively. For *O. mykiss*, hardness, alkalinity, and chloride ranged from 10 to 316 mg $CaCO_3$/L, from 4 to 196 mg $CaCO_3$/L, and from 1.8 to 98.4 mg/L, respectively. The sample size of the *H. azteca* ($n = 15$) and *O. mykiss* ($n = 21$) data sets from Pearcy et al. (2015) used in the bioavailability model development constrain the ability to reliably test for interactions. Nevertheless, the role of potential interaction effects between TMFs was explored and is included in Supporting Information, Table S3. This assessment also considered scenarios with a single pivot point in the *O. mykiss* data set removed (fluoride LC50 = 10.4 mg/L, $n = 20$).

Using the reported toxicity information from the acceptable acute studies (Supporting Information, Table S1), normalized acute toxicity effects measures were calculated for *H. azteca* and *O. mykiss* with the optimal linear models presented in Equations 1 and 2, respectively.

$$\begin{aligned} H.\ azteca\ LC_{50norm} = \exp[&\ln(LC_{50test}) + 0.218 \cdot [\ln(Hard_{test}) \\ &- \ln(Hard_{target})] - 0.545 \cdot [\ln(Cl_{test}) \\ &- \ln(Cl_{target})] - 0.277 \cdot [\ln(Alk_{test}) - \ln(Alk_{target})]] \end{aligned} \quad (1)$$

$$\begin{aligned} O.\ mykiss\ LC_{50norm} = \exp[&\ln(LC_{50test}) - 0.612 \cdot [\ln(Hard_{test}) \\ &- \ln(Hard_{target})] + 0.412 \cdot [\ln(Alk_{test}) - \ln(Alk_{target})]] \end{aligned} \quad (2)$$

In these equations, $LC50_{norm}$ is the normalized LC50 (milligrams per liter), $LC50_{test}$ is the reported acute fluoride LC50 (milligrams per liter), $Hard_{test}$ is the reported test hardness concentration (milligrams of $CaCO_3$ per liter), Cl_{test} is the reported test chloride concentration (milligrams per liter), Alk_{test} is the reported test alkalinity concentration in mg $CaCO_3$/L, $Hard_{target}$ is the hardness concentration to normalize to (milligrams per liter), Cl_{target} is the chloride concentration to normalize to (milligrams per liter), and Alk_{target} is the alkalinity concentration to normalize to (milligrams per liter). The *H. azteca* model (Equation 1) was applied to invertebrates to

normalize acute toxicity results, and the *O. mykiss* model (Equation 2) was applied to vertebrate fish to normalize acute toxicity results. Details of the normalization conditions are described as part of the FAV derivation and the empirical bioavailability model FAV sensitivity assessment. Normalization was not applied to amphibian taxa.

MLR model performance

Qualitative and quantitative methods were used to assess the model performance in addition to the standard diagnostic tools discussed already to help identify the optimal bioavailability models for *H. azteca* and *O. mykiss*. Visual methods included an analysis of modeled residuals against observed effect concentrations and TMFs as well as observed on predicted plots. Residual plots were used to understand how model residuals may be influenced by observed LC50 as well as each of the three TMFs (Supporting Information, Figures S1–S3). The predicted plots compared observed LC50 on predicted LC50 for both taxa after transformation from natural log units to standard units. We note that many empirical bioavailability models (Garman et al., 2020; Mebane et al., 2020) have been validated by plotting predicted effect concentrations on observed effect concentrations. Although the two approaches yield the same correlation coefficient, R^2, predicted on observed methods do not yield appropriate estimates of the slope and intercept, which are useful diagnostics for understanding model performance (Piñeiro et al., 2008). The VIFs calculated for the main effects of the top five candidate models were considered in the evaluation of model performance (Supporting Information, Table S4). In addition, the model performance scoring systems described by Garman et al. (2020) and Brix et al. (2021) were applied to the top five candidate models for *H. azteca* and *O. mykiss* (Supporting Information, Table S5). These metrics were used collectively to inform model selection and better understand the factors affecting model performance.

Species selection could not be conducted as part of the MLR model performance assessment because the taxa evaluated represent the extent of available toxicity data found within the literature. Both *H. azteca* and *O. mykiss* have been noted to be sensitive to fluoride toxicity across a gradient of water quality conditions (Camargo, 2003; McPherson et al., 2014; Pearcy et al., 2015). Interestingly, Soucek et al. (2015) demonstrated that the strain of *H. azteca* commonly used for aquatic toxicity testing in the United States may have a physiological dependence on chloride and that toxicity results obtained for this species using test waters with <15–20 mg/L chloride should be interpreted with caution because they may overestimate toxicity. These findings were considered as part of the FAV derivation and sensitivity analysis.

FAV derivation and empirical bioavailability model FAV sensitivity

The acute toxicity data set used to derive FAVs with consideration to TMFs was comprised of 16 species and 13 genera. Toxicity data for nine invertebrates, three fish, and one amphibian genera were available. For FAVs where TMFs were considered, normalization of Equations 1 and 2 was applied to invertebrate and fish taxa, respectively. The single amphibian taxon was not normalized prior to inclusion in FAV derivation.

The FAVs were estimated using the approach described by Stephan et al. (1985). The objective of this method is to estimate a statistical measure of the distribution of toxicity results that protect 95% of the aquatic genera tested. For each species with one or more acute value, species mean acute values (SMAVs) were calculated as the geometric mean of the normalized LC50 or the test reported LC50, specifically in the case of non-TMF FAV derivation, which is discussed in the following section. For a genus in which multiple SMAVs were calculated, the genus mean acute value (GMAV) was calculated as the geometric mean of the SMAVs. Details of example SMAV and GMAV calculations are provided in Supporting Information, Table S1. The resulting GMAVs are arranged in order of descending concentration (highest to lowest) and ranked where the lowest concentration is 1 and the highest concentration is rank n. Cumulative probability (p) is calculated according to a Weibull function where $p = R/(n+1)$. The four lowest GMAVs were selected to calculate the FAV, which was estimated based on the 5th percentile of cumulative probability distribution fit using a log-triangular model from Stephan et al. (1985) as follows:

$$S^2 = \left(\sum((\ln \text{GMAV})^2) - \left(\left(\sum(\ln \text{GMAV})\right)^2 / 4\right)\right) \Big/ \left(\sum(P) - \left(\left(\sum(\sqrt{P})\right)^2 / 4\right)\right) \quad (3)$$

$$L = \left(\sum(\ln \text{GMAV}) - S \cdot \left(\sum(\sqrt{P})\right)\right) / 4 \quad (4)$$

$$A = S \cdot (\sqrt{0.05}) + L \quad (5)$$

$$\text{FAV} = \exp(A) \quad (6)$$

In these equations, A predicts the 5th percentile FAV in units ln (fluoride [milligrams per liter]). The FAV is the exponent of Equation 5 in milligrams per liter of fluoride.

The sensitivity of FAVs was evaluated across a range of TMF concentrations that were similar to those used to inform the empirical MLR-based bioavailability models. This assessment was conducted to understand the relative importance of receptor groups across the range of TMFs and to constrain the direction and magnitude of anticipated changes to fluoride FAVs for which the MLR bioavailability models were built. Cumulative probability distributions of fluoride GMAVs normalized using the minimum and maximum TMF range of chloride and alkalinity were evaluated. The minimum and maximum TMFs ranged from 20 to 95 mg/L and from 16 to 108 mg $CaCO_3$/L for chloride and alkalinity, respectively. Hardness was assigned based on the hardness concentration that corresponds to the 10th and 90th percentile alkalinity to hardness ratio based on ratios derived for median surface water conditions throughout North America, presented in Supporting Information, Table S6. Hardness values of 22 and 47 mg

$CaCO_3$/L were assessed for the 16 mg $CaCO_3$/L alkalinity, and hardness values of 97 and 302 mg $CaCO_3$/L were assessed for the 108 mg $CaCO_3$/L alkalinity. These ranges were consistent with the TMFs that were used to derive the bioavailability models for alkalinity, hardness, and the maximum chloride. The minimum chloride range was established based on the findings presented by Soucek et al. (2015), which called into question the suitability of *H. azteca* toxicity data from the laboratory strain tested for use in FAV determination when chloride concentrations are <15–20 mg/L.

Non-TMF FAV derivation and comparison

The acute toxicity data set that was used to derive the non-TMF FAVs was comprised of 29 species and 24 genera. Toxicity data for genera in invertebrate ($n = 15$), fish ($n = 8$), and amphibian ($n = 1$) receptor groups were considered. Acute non-TMF FAVs were estimated using the approach described previously; however, test LC50 concentrations were not normalized using Equation 1 or 2. Acute *H. azteca* toxicity data included in the FAV assessment contained chloride concentrations ≥18.8 mg/L, in accordance with the findings of Soucek et al. (2015).

Further sensitivity analysis was conducted across the entire range of TMF variables to understand the influence of TMFs on FAVs. To understand how changes in TMF chemistry affect resulting FAVs, a sensitivity analysis was carried out for four chloride concentrations (20, 45, 70, and 95 mg/L) and four alkalinity concentrations (16, 47, 77, and 108 mg $CaCO_3$/L) across a continuous range of hardness values. The TMF normalization of FAVs was carried out iteratively using the R program. Source code developed by the USEPA (2018) was adapted to calculate FAVs for multiple TMF water quality input parameters.

ACR calculation and non-TMF FCV derivation and comparison

An analysis performed to understand how TMFs affect fluoride ACRs is provided in Supporting Information, Figure S5. This assessment identified six taxa ($n = 3$ fish and $n = 3$ invertebrates) suitable for ACR calculation where chronic and acute data were obtained from the same study using the same test water or where test water was most similar. Preference was given for chronic tests where the effect concentration was reported statistically (e.g., ECx) over the use of maximum-acceptable-toxicant concentrations (MATCs), lowest-observed-effect concentrations (LOECs), or no-observed-effect concentrations (NOECs). Details of the selection process are summarized in Supporting Information, Table S7. The ACR value used to derive FCVs was calculated as the geometric mean of the ACRs from the six taxa.

The chronic toxicity data set was also used to derive the non-TMF FCVs. This data set was comprised of 19 species and 19 genera (Supporting Information, Table S2). Toxicity data for genera in the invertebrate ($n = 7$), fish ($n = 6$), and algal ($n = 6$) receptor groups were considered. The nonlethal effect tests were prioritized in the order EC10, EC25, MATC, NOEC, and LOEC. Details of the selection process are provided in Supporting Information, Table S2. Non-TMF FCVs were estimated for genera using the approach described previously. Chronic *H. azteca* toxicity data included in the non-TMF FCV assessment contained chloride concentrations ≥18.8 mg/L, in accordance with the findings of Soucek et al. (2015).

RESULTS

MLR model performance

The top five empirical acute fluoride bioavailability models for *H. azteca* and *O. mykiss* considering main effects are provided in Table 1. The Rank 1 model for *H. azteca* was predicted by hardness, alkalinity, and chloride TMFs; and the Rank 1 model for *O. mykiss* LC50 was predicted by hardness and alkalinity TMFs. Figure 1 illustrates the predicted plots for both Rank 1 models. A summary of qualitative and quantitative performance metrics is provided as follows for each species.

For *H. azteca*, four of the five models had adjusted R^2 values >70% of the variance. The predicted R^2 was greatest in the Rank 1 model (0.80), which also had the lowest BIC (1.1). The

TABLE 1: Summary of the top five empirical acute fluoride bioavailability models by species ranked by model preference

Model	Rank	R^2	Adj. R^2	Pred. R^2	RSE	p	BIC	ΔBIC
Hyalella azteca								
$ln(LC50) = 1.573 - 0.218 \times ln(Hard) + 0.545 \times ln(Cl) + 0.277 \times ln(Alk)$	1	0.900	0.873	0.796	0.187	0.000	1.1	3.6
$ln(LC50) = 1.127 + 0.445 \times ln(Cl) + 0.213 \times ln(Alk)$	2	0.848	0.823	0.725	0.220	0.000	4.7	1.7
$ln(LC50) = 1.715 + 0.498 \times ln(Cl)$	3	0.797	0.781	0.666	0.245	0.000	6.4	0.8
$ln(LC50) = 2.117 - 0.142 \times ln(Hard) + 0.573 \times ln(Cl)$	4	0.821	0.791	0.670	0.240	0.000	7.2	17.6
$ln(LC50) = 1.444 + 0.479 \times ln(Alk)$	5	0.304	0.251	0.132	0.454	0.033	24.8	1.3
Oncorhynchus mykiss								
$ln(LC50) = 3.024 + 0.612 \times ln(Hard) - 0.412 \times ln(Alk)$	1	0.371	0.301	0.178	0.529	0.015	41.8	1.5
$ln(LC50) = 3.153 + 0.254 \times ln(Hard)$	2	0.219	0.177	0.073	0.574	0.033	43.3	1.5
$ln(LC50) = 3.012 + 0.593 \times ln(Hard) + 0.031 \times ln(Cl) - 0.41 \times ln(Alk)$	3	0.373	0.262	0.054	0.544	0.043	44.8	0.7
$ln(LC50) = 4.187$	4	0.000	0.000	−0.102	0.633	NA	45.5	0.5
$ln(LC50) = 3.632 + 0.201 \times ln(Cl)$	5	0.113	0.067	−0.077	0.612	0.136	46.0	0.3

R^2 = coefficient of determination; Adj. R^2 = adjusted R^2; Pred. R^2 = predicted R^2; RSE = residual standard error; BIC = Bayesian information criterion; ΔBIC = difference between BIC of rank n and rank $n+1$; LC50 = fluoride 50% lethal concentration, milligrams per liter; Hard = hardness, milligrams of $CaCO_3$ per liter; Alk = alkalinity, milligrams of $CaCO_3$ per liter.

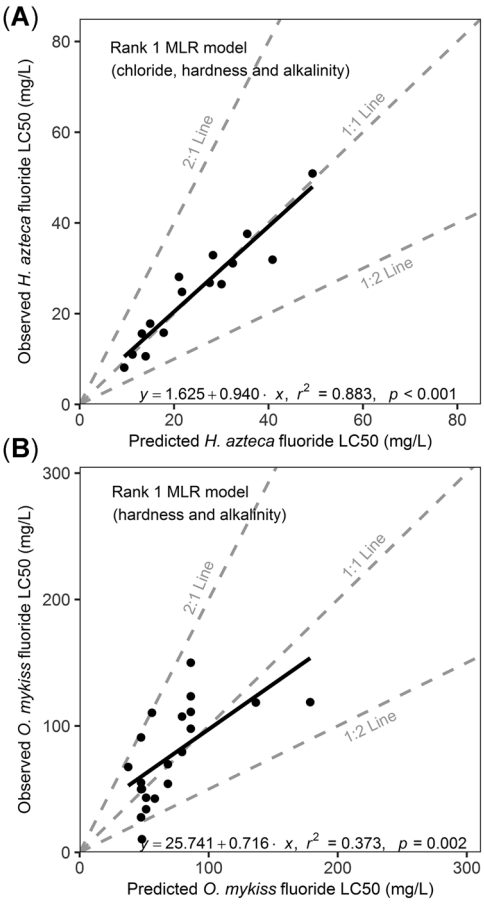

FIGURE 1: Scatterplot illustrating relationship between observed and predicted acute 50% lethal concentration for optimal (**A**) *Hyalella azteca* and (**B**) *Oncorhynchus mykiss* multiple linear regression models; solid line illustrates linear model fit, and dashed lines denote 2:1, 1:1, and 1:2 lines for reference. MLR = multiple linear regression; LC50 = 50% lethal concentration.

VIFs for the Rank 1 model were <2 for each parameter, which indicates limited collinearity between TMFs (Supporting Information, Table S4). The Rank 1 *H. azteca* model had positive alkalinity and hardness parameter coefficients and negative parameter coefficients for hardness. Residual plots exhibited low slopes, indicating that the predictors do not correlate nonlinearly with toxicity (Supporting Information, Figure S1). The Rank 1 model had a mean performance score of 0.97 based on the approach described by Garman et al. (2020) and 0.96 based on the approach described in Brix et al. (2021; Supporting Information, Table S5).

The predicted Rank 1 *H. azteca* model explained 88% of the variance in observed *H. azteca* LC50 values (Figure 1A; $R^2 = 0.88$). The observed versus predicted linear model slope was 0.94, with an intercept of 1.63, across $n = 15$ observations. The predictive model aligned strongly with the observations and the 1:1 line. Based on the qualitative and quantitative performance assessment, the Rank 1 *H. azteca* model was considered optimal. Potential interaction effects of the *H. azteca* model were explored but not considered further because of the strong performance of the main effects model (Supporting Information, Figure S2).

For *O. mykiss*, the adjusted R^2 was greatest for the Rank 1 model (0.30), and the predictive ability decreased as model rank number increased (Table 1). The VIFs for the Rank 1 model were 3.8 for both hardness and alkalinity. The VIFs were slightly greater than the threshold of 3 used to assess potential multicollinearity (Zuur et al., 2010; Supporting Information, Table S4). The Rank 1 *O. mykiss* model had negative alkalinity and positive hardness parameter coefficients. The differing parameter coefficients for the *O. mykiss* model are notable and explored in detail in the *Discussion* section. The observed VIFs were the result of the alkalinity and hardness treatments used by Pearcy et al. (2015). Residual plots exhibited low slopes for each TMF, indicating an absence of potential systematic bias in the *O. mykiss* Rank 1 model (Supporting Information, Figure S1). The residuals plotted against observed *O. mykiss* LC50 results illustrated that a pivot point was present where the observed fluoride LC50 (10.4 mg/L) was 4.6 times less than the predicted fluoride LC50. Removal of this point did not substantially change the model parameterization or coefficient estimates (Supporting Information, Table S3). Further review of model runs considering potential interaction effects resulted in marginal improvement to the *O. mykiss* model (Supporting Information, Table S3), and the Rank 1 main effects model was retained for use in subsequent FAV estimation. The Rank 1 model considering main effects had a mean performance score of 0.81 for both the approach by Garman et al. (2020) and the approach by Brix et al. (2021).

The predicted Rank 1 *O. mykiss* model explained 37% of the variance in observed *O. mykiss* LC50 values (Figure 1B; $R^2 = 0.37$). The observed versus predicted linear model slope was 0.71, with an intercept of 25.7, across $n = 21$ observations. Observed *O. mykiss* LC50 values about the low end of the effect concentration range were variable but generally within a factor of 2 of the predicted LC50.

Model-averaged importance of terms for the optimal *H. azteca* model and the *O. mykiss* model were chloride > alkalinity > hardness and hardness > alkalinity, respectively. The importance of chloride in the *H. azteca* model is apparent based on the ΔBIC between the Rank 4 and Rank 5 models. Rank 4 and Rank 5 mark the transition point between models that contain chloride and models that do not contain chloride for *H. azteca* (Table 1). The ΔBIC of 17.6 indicates that there is strong evidence for inclusion of chloride in candidate models. The optimal *O. mykiss* model did not include chloride as a predictive variable. In summary, the results of the model performance assessment indicate that the performance indices were slightly greater for the Rank 1 *H. azteca* model than the Rank 1 *O. mykiss* model. Nevertheless, the TMF and fluoride toxicity data from Pearcy et al. (2015) were sufficiently robust to develop MLR models for *H. azteca* and *O. mykiss*.

FAV derivation and empirical bioavailability model FAV sensitivity

The empirical bioavailability models had a pronounced effect on the distribution of normalized GMAV toxicity data across the range of TMFs tested (Figure 2). For each ranked

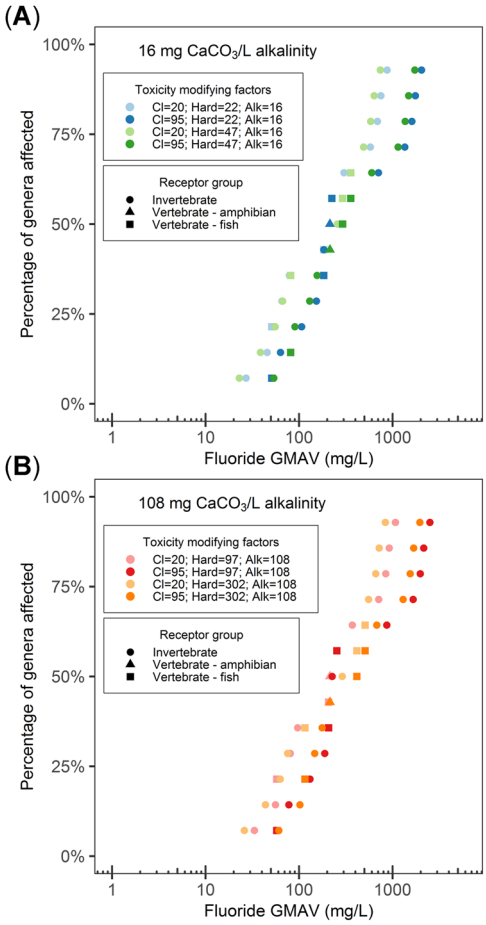

FIGURE 2: Cumulative probability distribution indicating the percentage of genera affected and fluoride genus mean acute values by receptor group and normalization based on the minimum and maximum toxicity-modifying factor concentrations (Cl = 20 and 95 mg/L; hardness = 12 and 302 mg $CaCO_3$/L; alkalinity = 16 and 116 mg $CaCO_3$/L). Hard = hardness; Alk = alkalinity; GMAV = genus mean acute value.

taxon in the cumulative probability distribution, the sensitivity in fluoride GMAV differed by a factor of approximately 7 when minimum and maximum TMF normalization approaches were applied. Invertebrates were the four most sensitive taxa in two of the eight TMF scenarios evaluated where alkalinity to hardness ratios were greatest (chloride = 20 mg/L, hardness = 47 mg $CaCO_3$/L, alkalinity = 16 mg $CaCO_3$/L and chloride = 20 mg/L, hardness = 302 mg $CaCO_3$/L, alkalinity = 108 mg $CaCO_3$/L). The four most sensitive taxa in the other scenarios contained a mix of fish and invertebrates. A similar range of estimated FAVs was observed in the 16 mg $CaCO_3$/L alkalinity scenario (Figure 2A) as the 108 mg $CaCO_3$/L alkalinity scenario (Figure 2B). However, greater sensitivity to FAVs was observed at low chloride when alkalinity was greater. The inclusion of the chloride term in the invertebrate model had a pronounced effect on the estimated FAVs, given the greater prevalence of invertebrates as the four most sensitive taxa. This evaluation indicates that a high degree of sensitivity exists between the response of invertebrates and fish to the empirical bioavailability models across a range of TMFs. The predicted response by taxa group is not consistent between models, which will affect the FAV derivation. Therefore, a more detailed sensitivity analysis of FAV response is described in the following section.

Non-TMF FAV derivation and comparison

Non-TMF FAVs were derived to understand basal conditions in the absence of water TMF normalization. Figure 3 illustrates the cumulative probability distribution of GMAVs when TMFs were not considered. In the absence of consideration to empirical models of bioavailability, the non-TMF FAV was 30.6 mg/L fluoride. The four most sensitive genera in order of decreasing sensitivity were the invertebrates *Hyalella* > *Hexagenia* > *Hydropsyche* > *Chimara*. *Hyalella* toxicity results at low-chloride test conditions were not included in the assessment, yet it remained the most sensitive taxon in the cumulative distribution. Salmonid taxa (subfamily Salmoninae) exhibited the greatest sensitivity of the fish species evaluated. Sunfish (Centrarchidae) and topminnow (Poeciliidae) fish were among the most tolerant. Mean hardness, alkalinity, and chloride for the toxicity tests included in the cumulative genera probability distribution were 106.0 mg $CaCO_3$/L, 58.0 mg $CaCO_3$/L, and 19 mg/L, respectively.

A sensitivity analysis was performed to constrain the direction and magnitude of anticipated changes to fluoride FAVs across ranges of chloride, alkalinity, and hardness values for which the MLR bioavailability models were built. The results of this sensitivity analysis for four discrete chloride concentrations and four discrete alkalinity concentrations across a continuous range of hardness values are presented in Figure 4. The hardness concentrations corresponding to the 90th and 10th percentile alkalinity to hardness ratios associated with each alkalinity concentration are highlighted to better inform the applicable sensitivity analyses to North American surface waters. Chloride was found to be directly proportional to the FAV across the modeled range of alkalinity and hardness. Alkalinity was positively related to the FAV at the moderate to high end

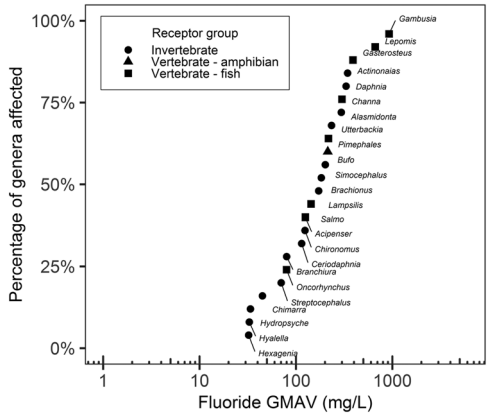

FIGURE 3: Cumulative probability distribution of percentage of genera affected on fluoride genus mean acute values used to derive non–toxicity-modifying factor fluoride final acute value = 30.6 mg/L. GMAV = genus mean acute value.

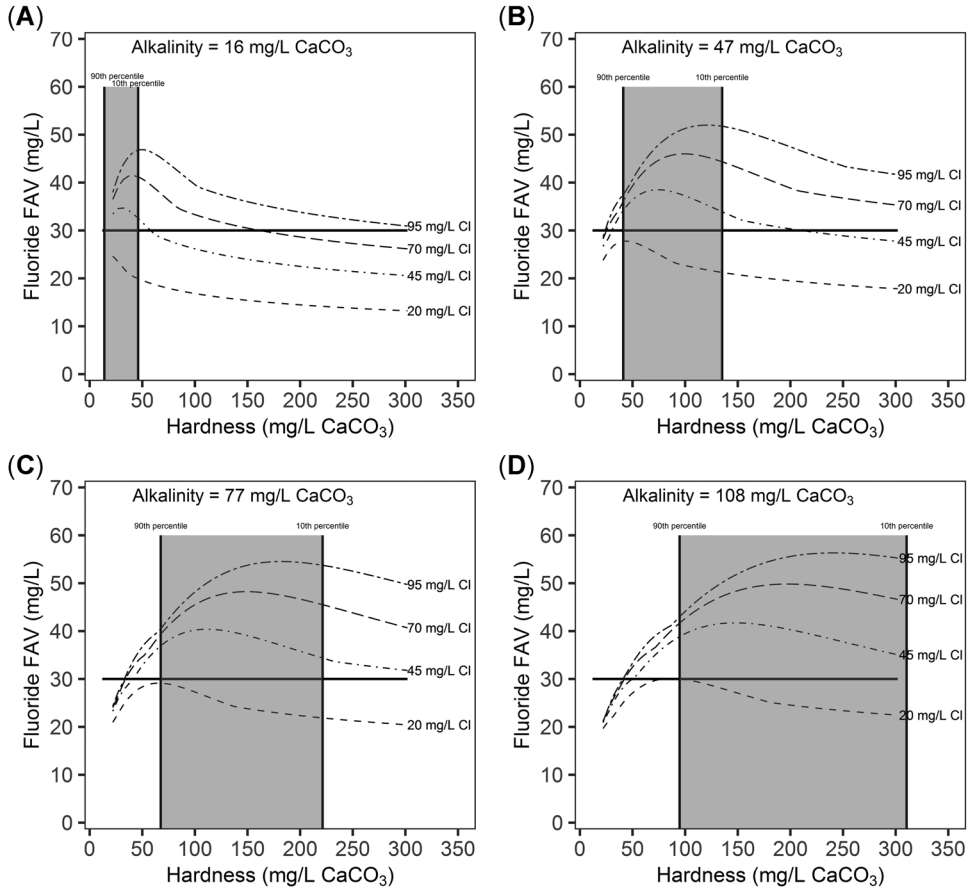

FIGURE 4: Multiplot illustrating the sensitivity of fluoride final acute values (FAVs) based on hardness for four different alkalinity concentrations (**A**–**D**); dashed lines denote chloride concentration, and the solid horizontal line represents the non–toxicity-modifying factor fluoride FAV; gray shading illustrates the 90th to 10th percentile range of median hardness concentrations that correspond to each alkalinity concentration.

of the hardness range but negatively related at lower hardness values. Modeled FAV sensitivity to hardness generally increased with increasing chloride and alkalinity.

The greatest FAV was 56.3 mg/L fluoride when chloride, alkalinity, and hardness were 95 mg/L, 108 mg/L $CaCO_3$, and 242 mg/L $CaCO_3$, respectively (Figure 4F and Table 2). Conversely, the lowest fluoride FAV was 13.2 mg/L when chloride, alkalinity, and hardness were 20 mg/L, 16 mg/L $CaCO_3$, and 302 mg/L $CaCO_3$, respectively (Figure 4A). It should be noted, however, that this combination of water quality parameters represents an unrealistic scenario for the calculated lowest acute toxicity for fresh surface waters in North America. Because carbonate bases and alkaline earth metals are derived from the same geogenic sources, concentrations of hardness

TABLE 2: Summary of acute and chronic final values by derivation type and associated toxicity-modifying factor water quality conditions

Derivation type	Chloride (mg/L)	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	ACR (unitless)	Fluoride final value (mg/L)
Acute–minimum TMF range[a]	20	72	16	–	18.1
Acute–maximum TMF range[b]	95	242	108	–	56.3
Acute non-TMF[c]	–	–	–	–	30.6
Chronic (ACR)–minimum TMF range[d]	20	132	16	5.4	3.4
Chronic (ACR)–maximum TMF range[e]	95	242	108	5.4	10.4
Chronic (ACR) non-TMF[f]	–	–	–	5.4	5.7
Chronic non-TMF[g]	–	–	–	–	4.0

[a]Calculated suitable minimum final acute value (FAV) using the empirical bioavailability modeling approach with consideration to TMFs.
[b]Calculated suitable maximum FAV using the empirical bioavailability modeling approach with consideration to TMFs.
[c]Calculated non-TMF FAV (does not consider TMFs).
[d]Calculated as a divided by an ACR of 5.4.
[e]Calculated as b divided by an ACR of 5.4.
[f]Calculated as c divided by an ACR of 5.4.
[g]Calculated non-TMF final chronic value (does not consider TMFs).
ACR = acute to chronic ratio; TMF = toxicity modifying factor.

and alkalinity tend to covary (Boyd et al., 2016). A meta-analysis of publicly available surface water data in North America revealed that the 5th percentile and the 95th percentile of freshwater systems exhibit an alkalinity to hardness ratio between 0.22 and 1.33 ($n = 2172$). Summary data and related information for this analysis are presented in Supporting Information, Table S6 and Figure S4. The lowest FAV within this range of alkalinity to hardness ratios would occur when chloride is 20 mg/L and the alkalinity to hardness ratio 0.22, the 5th percentile. Adopting these constraints in the sensitivity analysis results in a minimum FAV of 18.1 mg/L when chloride, alkalinity, and hardness were 20 mg/L, 16 mg/L $CaCO_3$, and 72 mg/L $CaCO_3$, respectively. Although the FAV of 18.1 mg/L represents the lower boundary of suitable FAVs when TMFs are considered, this scenario is still of low probability for freshwaters in North America.

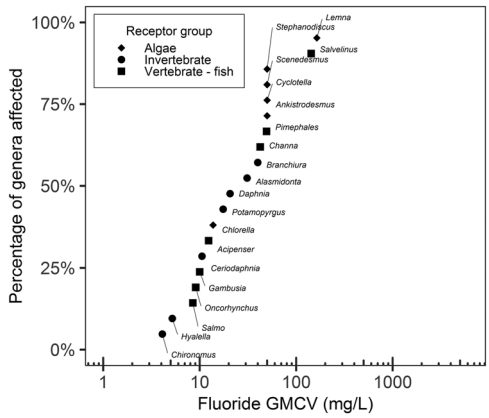

FIGURE 5: Cumulative probability distribution of percentage of genera affected on fluoride genus mean chronic values used to derive non–toxicity-modifying factor fluoride final chronic value = 4.0 mg/L. GMCV = genus mean chronic value.

ACR calculation and non-TMF FCV derivation and comparison

Species ACRs from the invertebrate and fish taxa ranged from 3.6 to 9.3. The resulting geometric mean ACR was 5.4. Details of an analysis performed to assess the effect of water quality TMF relative percent difference on the fluoride ACRs can be found in Supporting Information, Figure S5. The FCVs were estimated by dividing FAVs and the non-TMF FAV by the ACR. Using the ACR approach, the FCVs ranged from 3.4 to 10.4 mg/L when TMFs were considered. The FCV estimated from the non-TMF FAV was 5.7 mg/L fluoride. Table 2 summarizes acute and chronic protective values derived with and without consideration to TMFs. Non-TMF FCVs were estimated without application of the bioavailability model to understand basal conditions in the absence of water quality parameter normalization. Figure 5 illustrates the cumulative probability distribution of genus mean chronic values when TMFs were not considered. In the absence of consideration to empirical models of bioavailability, the non-TMF FCV was 4.0 mg/L fluoride. In order of decreasing sensitivity, the invertebrates *Chironomus* and *Hyalella* had the lowest chronic fluoride effect concentrations. Salmonid fish species (subfamily Salmoninae) *Salmo* and *Oncorhynchus* were the third and fourth most sensitive taxa, respectively. Algae were the most tolerant taxa in the cumulative probability distribution. Mean ± standard deviation hardness, alkalinity, and chloride for the toxicity tests included in the cumulative genera probability were 114.4 ± 80.5 mg $CaCO_3$/L, 52.9 ± 30.8 mg $CaCO_3$/L, and 17.6 ± 21.2 mg/L, respectively. Although some studies contained multiple treatments of water quality conditions for a given taxa, there was insufficient sample size to conduct an MLR approach on chronic toxicity data.

DISCUSSION

Comparison to prior MLR models

The summary of empirical metal bioavailability models presented by Brix et al. (2020) and others was used to assess the magnitude of TMF parameter estimates for other metals relative to our fluoride MLR models. Acute MLR models for copper and zinc had parameter coefficients ranging from 0.139 to 1.065 for ln(Hardness) depending on the receptor and analyte. These parameter coefficients aligned with our *O. mykiss* model, 0.642 × ln(Hardness), but not with that of the *H. azteca* model, which had an opposite parameter coefficient (−0.212 × ln[Hardness]). It should be noted that the MLR models for metals summarized by Brix et al. (2020) are parameterized to predict acute toxicity in units of micrograms per liter, whereas our model predicted fluoride LC50 in units of milligrams per liter. Alkalinity and chloride were not evaluated in any of the models presented by Brix et al. (2020). For some metals (e.g., copper), increased alkalinity and the presence of hydroxyl groups were found to form less toxic copper-base complexes (Pagenkopf et al., 1974; Stiff, 1971). Fulton and Meyer (2014) found alkalinity to be a stronger predictor of copper effect concentrations than hardness and other TMFs in acute (48 h) studies on *Daphnia magna*. In addition, Fulton and Meyer found that the hardness to alkalinity ratio was negatively correlated with *D. magna* effects and noted that use of the ratio may be problematic because it does not account for absolute concentrations of alkalinity. Camargo (2004) found that chloride term parameter estimates ranged from 0.17 to 0.43 when the log(effect) was predicted by log(chloride). The magnitude of the chloride slope estimates for the Rank 1 *H. azteca*, as well as the hardness and alkalinity, were similar to other TMFs applied. Greater consideration to the use of alkalinity and chloride in empirical bioavailability models should be made.

The adjusted R^2 of the empirical metal bioavailability models summarized by Brix et al. (2020) ranged from 0.55 to 0.97. Models with an adjusted $R^2 < 0.50$ were excluded from the summary. The optimal *H. azteca* model (adjusted $R^2 = 0.88$) was quite robust and occurred at the upper end of this range. The adjusted R^2 of the optimal *O. mykiss* model was lower than the range (adjusted $R^2 = 0.33$). The lower explanatory power is not anticipated to have a major effect on the resulting FAVs and FCVs because of the low number of fish taxa within the cumulative probability data sets.

The opposite parameter coefficients observed for hardness and alkalinity between the *H. azteca* and *O. mykiss* bioavailability models was noteworthy and likely influenced by a number of factors. The differing parameter coefficient response may be driven by differences in the underlying *H. azteca* and *O. mykiss* data sets presented by Pearcy et al. (2015). *Hyalella azteca* toxicity tests at the greatest alkalinity and hardness treatments did not meet control acceptability criteria and were not reported. These data points were included in the *O. mykiss* tests and contributed to the slight collinearity observed. The differing coefficients may also be attributed to differences in how receptor groups respond to TMFs, which is explored in the subsequent sections. Future replication of ecotoxicity tests across carefully selected water quality conditions is needed.

Abiotic mechanisms of toxicity amelioration

While determining the mechanisms of toxicity modification that hardness, alkalinity, and chloride exert on fluoride toxicity was not an objective of the present study, the sensitivity analysis performed does provide some insight into potential chemical interactions that may underpin the empirical relationships in the MLR. The fact that the fluoride FAV is directly proportional to the chloride concentration across the entire range of modeled hardness and alkalinity reinforces prior mechanistic studies that show that competition for biological uptake between free chloride anions and free fluoride anions inhibits the toxicity of fluoride. This "competing ion" mode of toxicity modification has been demonstrated to occur for various toxic metals and is a fundamental idea behind the biotic ligand model (Paquin et al., 2002). Competition between anions may also explain why the fluoride FAV generally increases with increasing alkalinity. Possible modes of toxicity modification exhibited by hardness are more difficult to elucidate but may be related to the formation of complexes between hardness cations and alkalinity bases that reduce the pool of anions available to compete with fluoride for uptake sites. The coefficient terms for hardness and alkalinity in the invertebrate MLR model are likely driving this observed pattern, which would explain the shift in maximum FAV to higher hardness concentrations with each increasing step in alkalinity (Figure 4). For example, as alkalinity increases, increasing concentrations of hardness cations are required to complex base ions that would otherwise compete with fluoride for biological uptake. Given a larger input data set, it is quite possible that these effects would be captured by a hardness and alkalinity interaction term; however, this was not possible in the present assessment because of limited sample size and limited variability in the alkalinity to hardness ratios selected for each treatment in the experiments conducted by Pearcy et al. (2015). Mechanistic studies are needed to better understand the interaction between alkalinity and hardness in bioavailability models and the ameliorating effect on toxicity to inorganic anions.

Fluoride toxicity mechanisms in fish

In fish, the specific mechanism for this amelioration of fluoride toxicity is somewhat uncertain. Giguère and Campbell (2004) hypothesized that three mechanisms could explain the ameliorating effect of hardness: (1) the test organism is benefiting from the presence of hardness cations (Ca^{2+}, Mg^{2+}), either externally, at epithelial membranes, or internally; (2) complexation between fluoride ions and hardness cations, which reduces the free fluoride concentration; and/or (3) precipitation of calcium fluoride (CaF_2) in aquatic media, which also reduces the effective fluoride concentration.

The optimized model for *O. mykiss* contained hardness and alkalinity but did not contain chloride as an explanatory variable. It is possible that chloride may not have been an important TMF in predicting fluoride toxicity because of the fry-stage fish used in toxicity testing by Pearcy et al. (2015). Neuhold and Sigler (1962) attributed the ameliorating effect of chloride on fluoride toxicity to adaptations linked to fish salinity tolerance. Salinity tolerance has been shown to increase at the metamorphic transition from larva to juvenile (Varsamos et al., 2005). The life stage of the test organisms used may explain some differences observed in the role of chloride to ameliorate fluoride toxicity and why chloride was not an explanatory variable in our model.

In addition, the *O. mykiss* MLR model parameter coefficients were positive for hardness and negative for alkalinity, which was the opposite of what was observed for the *H. azteca* model. The positive parameter coefficient for hardness is consistent with Fieser et al. (1986), Wright (1977), and others who found the presence of calcium to have a pronounced effect on decreasing toxicity to fish. The specific interaction between alkalinity and hardness has not been extensively studied in MLR frameworks for fish. Wurts and Perschbacher (1994) found increasing alkalinity at fixed hardness to increase mortality of channel catfish (*Ictalurus punctatus*) exposed to 28 mg/L $CuSO_4$. Wurts and Perschbacher (1994) concluded that alkalinity was the primary factor controlling *I. punctatus* toxicity.

Fluoride toxicity mechanisms in invertebrates

Anion competition at active sites within cells may explain the strong influence of chloride on the invertebrate model we developed. Camargo (2003) attributed the mechanism responsible for the reduced fluoride toxicity caused by chloride in invertebrates to greater competition for the same binding sites of the cytosolic side of the cell membrane. Mechanistically, this means that the likelihood that the cells incorporate chloride over fluoride increases with increasing chloride concentrations. For example, if the fluoride concentration is 10 times greater than the chloride concentration, fluoride will easily be transported into the cell, by means of anion transport pathways in the cell membrane, and enact an effect on the active site. If there is 10 times less fluoride than chloride, chloride will be the dominant anion present across the anion transport pathway capable of enacting an effect on the active site. Insects have specifically adapted chloride epithelia that transport ions to help facilitate osmoregulation (Komnick, 1977). Studies that have examined multiple sizes of invertebrates also note decreased toxicity to fluoride with increasing size, even in the presence of chloride. In invertebrates, this may also be attributed to greater osmoregulatory ability in more mature larval or

adult invertebrate life stages. A similar mechanism has been described for the toxicity of nitrite (NO_2^-) in the presence of increased chloride (Alonso & Camargo, 2008). In summary, the mechanisms acting on fluoride toxicity in aquatic organisms support the use of MLR-based empirical bioavailability models including chloride and hardness.

Although mechanistic processes support the observed influence of TMFs on fluoride toxicity, the ameliorating effect induced by other cations and anions has not been fully evaluated. Research is limited on what mechanism drives the effect of alkalinity. Decreased toxicity at increased alkalinity may be driven by the greater incidence of hydroxyl anions or other alkaline bases by a similar mechanism as the chloride anion or through complexation. The observed difference in parameter coefficients between fish and invertebrates supports possible different modes of action for ameliorating effects between organism groups and warrants further research. This is an interesting observation because hardness and alkalinity correlate well in natural waters. Previous empirical and mechanistic bioavailability models that relied on hardness as a predictive variable may not fully account for the ameliorating capacity of alkalinity, especially at high alkalinity to hardness ratios. It is recommended that the interaction between hardness and alkalinity in MLR models and the importance of alkalinity as an ameliorating factor be evaluated further in freshwater organisms.

Complexities and logistical constraints associated with large factorial experimental designs present challenges to fully assessing the interaction between mechanistic and empirical bioavailability models. Few toxicological studies evaluate the full suite of base cations and anions needed to elucidate these interactions. Targeted toxicity testing for fluoride across a broad range of taxa and TMFs is recommended to further refine and validate suitable empirical bioavailability models.

Considerations for species selection

The freshwater amphipod *H. azteca* has been maintained as a model laboratory organism for aquatic toxicology studies because of its sensitivity to a wide variety of substances. However, the homogeneity of different laboratory cultures of this species in laboratories across North America has been called into question. Major et al. (2013) performed a genetic analysis of *H. azteca* species from 15 laboratories in the United States and Canada and 22 field sites located east of the Mississippi River and found six well-supported divergent clades of *H. azteca*. Each of these six clades was represented by organisms collected from field sites, yet only two clades were represented in laboratory cultures (US Lab clade and Burlington clade). Of the 15 laboratory cultures sampled by Major et al. (2013), 14 were identified as the US Lab clade. This clade was only found in four of the 22 field collection sites sampled, all of which were located in northern Florida. These results suggest that organisms used for laboratory toxicity testing may not be representative of wild populations of *H. azteca* in North America. Soucek et al. (2015) performed a series of toxicity tests on the US Lab clade and the Burlington clade and found that growth and reproduction of the US Lab clade exhibited a strong dependence on chloride concentration, even in the absence of a toxicant, while the Burlington clade showed no such relationship. Soucek et al. (2015) concluded that the US Lab clade may have a physiological requirement for chloride and that toxicity results generated with the US Lab clade of *H. azteca* in test waters with <15–20 mg/L chloride should be interpreted with caution because they may overestimate toxicity. Additional focused testing of other invertebrate species that are not known to exhibit this low chloride sensitivity is warranted.

Pearcy et al. (2015) found chloride to be the major TMF for fluoride in acute toxicity tests for *H. azteca* individuals obtained from Aquatic Biosystems, representing the US Lab clade. Of the 19 test water conditions used by Pearcy et al. (2015) for *H. azteca*, nine contained a chloride concentration of <20 mg/L. In light of the findings from Soucek et al. (2015), it is unclear if these results indicate sensitivity of *H. azteca* to fluoride or if they reflect the sensitivity of the US Lab clade to low-chloride conditions. In either case, these results should be interpreted with caution until similar toxicity testing can be carried out using the Burlington clade of *H. azteca* or another representative taxon determined through a species selection process. These findings were the basis for the exclusion of modeled fluoride FAVs at chloride test concentrations <20 mg/L in the present study. Further research is warranted to determine the bioavailability (and thus the toxicity) of fluoride in the presence of low chloride concentrations.

Recent studies that utilized species sensitivity distribution approaches to derive non-TMF FCVs have likely overestimated suitable benchmarks because of the influence of species selection and observed sensitivity to *H. azteca*. McPherson et al. (2014) identified a chronic freshwater benchmark of 1.9 mg/L fluoride; however, this result was strongly influenced by inconsistent consideration of species selection and alignment with stated methods. Specifically, the numeric approach discussed in the methods presented by McPherson et al. (2014) using the geometric mean to calculate species mean chronic values was not consistently applied in the resulting species sensitivity distribution. The minimum toxicity result for *H. azteca* (1.8 mg/L) was used as the SMAV instead of the geometric mean 10% inhibition concentration (IC10) of 3.3 mg/L. Our non-TMF FCV of 4.0 mg/L fluoride was informed by an *H. azteca* IC10 of 5.2 mg/L, which accounts for the known sensitivity of the receptor at low chloride (Soucek et al., 2015). This highlights the need for detailed review and understanding of the toxicological data supporting derived protective values, particularly the species selection for sensitive taxa.

Application and limitations of FAVs

The FAVs and non-TMF FAVs we developed aligned with existing estimates of FAVs that considered univariate approaches with hardness. The FAVs ranged from 18 to 64 mg/L fluoride depending on the distribution of TMFs, and the non-TMF FAV was 30.6 mg/L. The FAVs derived across the same hardness range in Illinois, Michigan, and New York ranged from

3.2 to 18.3 mg/L, from 11.3 to 20.1 mg/L, and from 1.5 to 28.9 mg/L fluoride, respectively. The British Columbia univariate LC50 model ranged from 48.2 to 177.8 mg/L fluoride. Our MLR models were bounded with the water chemistry TMFs that have been used in the US and British Columbian models. Because our amphipod model was constrained to chloride concentrations >20 mg/L, additional work is needed to better understand the suitability of these limits in freshwaters with low chloride concentrations. However, it should be noted that many large riverine systems in the United States and elsewhere, globally, have chloride concentrations >20 mg/L.

The FCVs (3.4–10.4 mg/L fluoride) and non-TMF FCV (4.0 mg/L fluoride) we developed indicate that existing FCVs, such as those promulgated in British Columbia, Illinois, Michigan, and North Carolina, are likely overly conservative. As mentioned, the FCV developed by McPherson et al. (2014) was estimated at a lower concentration because of the use of the species minimum acute value for *H. azteca* instead of the SMAV and the absence of consideration to species selection for sensitive taxa. Other state-specific guidelines in the United States and interim guidance recommended in Canada are also overly conservative, which is primarily attributed to the application of unrealistic assessment factors or ACRs. For example, the CCME (2002) bases its interim chronic fluoride criteria on one 144-h LC50 toxicity test result for *Hydropsyche bronta* multiplied by an assessment factor of 0.01. Similarly, the state of North Carolina bases its current chronic fluoride limit of 1.8 mg/L on a single acute toxicological test for rainbow trout (LC50 = 36.2 mg/L fluoride) divided by an ACR of 20, which our study demonstrates is outside the range of appropriate ACRs for fluoride. Therefore, the present study highlights the efficacy of MLR approaches for the inorganic ion fluoride and emphasizes the need for greater consideration to the role of TMFs in both acute and chronic protective value determination for effective fluoride management in surface water.

CONCLUSION

In conclusion, the present study demonstrates the utility of MLR-based empirical bioavailability modeling to enhance the derivation of acute and chronic protective values for fluoride. Our model includes important TMFs that are known to affect the toxicity of fluoride to freshwater receptors. The behavior of the models aligns with mechanisms known to affect the bioavailability of fluoride exposure. The specific mechanisms controlling the role of alkalinity and hardness toxicity modification offer exciting opportunities for future fluoride aquatic ecotoxicity research. Careful consideration of both TMFs is needed for bioavailability models for other constituents. Nevertheless, the application of empirical models in FAV derivation highlights that existing fluoride protective values are likely overly conservative, especially when TMFs that mitigate the toxicity of fluoride are at the upper end of their respective distributions. For FAVs, this is attributed to an absence of considerations of important TMFs, notably chloride and alkalinity, which reduces the effects of fluoride at the active site. The present study found that at >20 mg/L chloride, the preliminary FAVs for fluoride were within 1 order of magnitude and ranged from approximately 18 to 56 mg/L, depending on water chemistry. Non-TMF FAVs were 30.6 mg/L fluoride when TMFs were not considered. Existing FCVs may also be overly conservative because of the application of unrealistic ACR estimates, the use of application factors, or the inclusion of overly sensitive taxa in protective value derivation approaches. Our assessment indicated that suitable FCVs would range from 3.4 to 10.4 mg/L fluoride. Depending on the presence of important TMFs, FCVs could be several times greater than existing limits. Species selection should be considered carefully when developing empirical bioavailability models to control for experimental conditions unrelated to fluoride exposure. Research is being considered to expand on this existing empirical framework and provide additional confirmatory assessment of bioavailability models across a greater number of taxa and range of water quality conditions.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5259.

Acknowledgment—We thank the International Aluminium Institute member companies and the Aquatic Fluoride Working Group for the collaboration and technical contributions to the present study. We also thank an anonymous reviewer, M.-C. Simard, and W. Adams who provided insightful comments that helped clarify and strengthen the present study. This material is based on work supported by the International Aluminium Institute, Alcoa Corporation, and EHS Support.

Disclaimer—The present study presents the views and opinions of its authors and not those of the International Aluminium Institute, EHS Support, or Alcoa Corporation.

Author Contributions Statement—Samuel Parker and Austin Wilkes were responsible for the document preparation, data and statistical analyses, and figure generation. Gary Long, Nigel W. E. Goulding, and Rajat Ghosh contributed to the study design, document framing, technical review, and editing.

Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (samuel.parker@ehs-support.com).

REFERENCES

Adams, W., Blust, R., Dwyer, R., Mount, D., Nordheim, E., Rodriguez, P. H., & Spry, D. (2020). Bioavailability assessment of metals in freshwater environments: A historical review. *Environmental Toxicology and Chemistry*, *39*(1), 48–59. https://doi.org/10.1002/etc.4558

Aho, K., Derryberry, D., & Peterson, T. (2014). Model selection for ecologists: The worldviews of AIC and BIC. *Ecology*, *95*(3), 631–636. https://doi.org/10.1890/13-1452.1

Alonso, A., & Camargo, J. A. (2008). Ameliorating effect of chloride on nitrite toxicity to freshwater invertebrates with different physiology: A comparative study between amphipods and planarians. *Archives of Environmental Contamination and Toxicology*, *54*(2), 259–265. https://doi.org/10.1007/s00244-007-9034-0

Baker, J. A., Gilron, G., Chalmers, B. A., & Elphick, J. R. (2017). Evaluation of the effect of water type on the toxicity of nitrate to aquatic organisms. *Chemosphere*, *168*, 435–440. https://doi.org/10.1016/j.chemosphere.2016.10.059

Boyd, C. E., Tucker, C. S., & Somridhivej, B. (2016). Alkalinity and hardness: Critical but elusive concepts in aquaculture. *Journal of the World Aquaculture Society*, *47*(1), 6–41. https://doi.org/10.1111/jwas.12241

Brix, K. V., Deforest, D. K., Tear, L., Grosell, M., & Adams, W. J. (2017). Use of multiple linear regression models for setting water quality criteria for copper: A complementary approach to the biotic ligand model. *Environmental Science & Technology*, *51*(9), 5182–5192. https://doi.org/10.1021/acs.est.6b05533

Brix, K. V., DeForest, D. K., Tear, L., Peijnenburg, W., Peters, A., Middleton, E. T., & Erickson, R. (2020). Development of empirical bioavailability models for metals. *Environmental Toxicology and Chemistry*, *39*(1), 85–100. https://doi.org/10.1002/etc.4570

Brix, K. V., Tear, L., Santore, R. C., Croteau, K., & DeForest, D. K. (2021). Comparative performance of multiple linear regression and biotic ligand models for estimating the bioavailability of copper in freshwater. *Environmental Toxicology and Chemistry*. https://doi.org/10.1002/etc.5012

Brix, K. V., Volosin, J. S., Adams, W. J., Reash, R. J., Carlton, R. G., & McIntyre, D. O. (2001). Effects of sulfate on the acute toxicity of selenate to freshwater organisms. *Environmental Toxicology and Chemistry*, *20*(5), 1037–1045. https://doi.org/10.1002/etc.5620200514

Calcagno, V., & de Mazancourt, C. (2010). glmulti: An R package for easy automated model selection with (generalized) linear models. *Journal of Statistical Software*, *34*(12), 1–29. https://doi.org/10.18637/jss.v034.i12

Camargo, J. A. (2003). Fluoride toxicity to aquatic organisms: A review. *Chemosphere*, *50*(3), 251–264. https://doi.org/10.1016/S0045-6535(02)00498-8

Camargo, J. A. (2004). Effects of body size and sodium chloride on the tolerance of net-spinning caddisfly larvae to fluoride toxicity. *Bulletin of Environmental Contamination and Toxicology*, *72*(3), 579–585. https://doi.org/10.1007/s00128-004-0283-6

Canadian Council of Ministers of the Environment. (2002). Fluoride [Fact sheet]. https://ccme.ca/en/summary-table?lang=en%26factsheet=98526pdf=1

DeForest, D. K., Brix, K. V., Tear, L. M., & Adams, W. J. (2018). Multiple linear regression models for predicting chronic aluminum toxicity to freshwater aquatic organisms and developing water quality guidelines. *Environmental Toxicology and Chemistry*, *37*(1), 80–90. https://doi.org/10.1002/etc.3922

DeForest, D. K., Brix, K. V., Tear, L. M., Cardwell, A. S., Stubblefield, W. A., Nordheim, E., & Adams, W. J. (2020). Updated multiple linear regression models for predicting chronic aluminum toxicity to freshwater aquatic organisms and developing water quality guidelines. *Environmental Toxicology and Chemistry*, *39*(9), 1724–1736. https://doi.org/10.1002/etc.4796

Di Toro, D. M., Allen, H. E., Bergman, H. L., Meyer, J. S., Paquin, P. R., & Santore, R. C. (2001). Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environmental Toxicology and Chemistry*, *20*(10), 2383–2396. https://doi.org/10.1002/etc.5620201034

Elphick, J. R. F., Bergh, K. D., & Bailey, H. C. (2011). Chronic toxicity of chloride to freshwater species: Effects of hardness and implications for water quality guidelines. *Environmental Toxicology and Chemistry*, *30*(1), 239–246. https://doi.org/10.1002/etc.365

Elphick, J. R. F., Davies, M., Gilron, G., Canaria, E. C., Lo, B., & Bailey, H. C. (2011). An aquatic toxicological evaluation of sulfate: The case for considering hardness as a modifying factor in setting water quality guidelines. *Environmental Toxicology and Chemistry*, *30*(1), 247–253. https://doi.org/10.1002/etc.363

Fieser, A. H., Sykora, J. L., Kostalos, M. S., Wu, Y. C., & Weyel, D. W. (1986). Effect of fluorides on survival and reproduction of *Daphnia magna*. *Journal of the Water Pollution Control Federation*, *58*(1), 82–86. http://www.jstor.org/stable/25042845

Fulton, B. A., & Meyer, J. S. (2014). Development of a regression model to predict copper toxicity to *Daphnia magna* and site-specific copper criteria across multiple surface-water drainages in an arid landscape. *Environmental Toxicology and Chemistry*, *33*(8), 1865–1873. https://doi.org/10.1002/etc.2631

Garman, E. R., Meyer, J. S., Bergeron, C. M., Blewett, T. A., Clements, W. H., Elias, M. C., & Ryan, A. C. (2020). Validation of bioavailability-based toxicity models for metals. *Environmental Toxicology and Chemistry*, *39*(1), 101–117. https://doi.org/10.1002/etc.4563

Giguère, A., & Campbell, P. G. C. (2004). Fluoride toxicity towards freshwater organisms and hardness effects—Review and reanalysis of existing data. *Revue des Sciences de l'Eau*, *17*(3), 373–393. https://doi.org/10.7202/705539ar

Gillio Meina, E., Niyogi, S., & Liber, K. (2020). Multiple linear regression modeling predicts the effects of surface water chemistry on acute vanadium toxicity to model freshwater organisms. *Environmental Toxicology and Chemistry*, *39*(9), 1737–1745. https://doi.org/10.1002/etc.4798

Ji, J., He, E., Qiu, H., Peijnenburg, W. J. G. M., Van Gestel, C. A. M., & Cao, X. (2020). Effective modeling framework for quantifying the potential impacts of coexisting anions on the toxicity of arsenate, selenite, and vanadate. *Environmental Science & Technology*, *54*(4), 2379–2388. https://doi.org/10.1021/acs.est.9b06837

Komnick, H. (1977). Chloride cells and chloride epithelia of aquatic insects. *International Review of Cytology*, *49*, 285–329. https://doi.org/10.1016/S0074-7696(08)61951-8

Lazorchak, J. M., & Smith, M. E. (2007). Rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) 7-day survival and growth test method. *Archives of Environmental Contamination and Toxicology*, *53*(3), 397–405. https://doi.org/10.1007/s00244-006-0227-8

Major, K., Soucek, D. J., Giordano, R., Wetzel, M. J., & Soto-Adames, F. (2013). The common ecotoxicology laboratory strain of *Hyalella azteca* is genetically distinct from most wild strains sampled in eastern North America. *Environmental Toxicology and Chemistry*, *32*(11), 2637–2647. https://doi.org/10.1002/etc.2355

McPherson, C. A., Lee, D. H. Y., & Chapman, P. M. (2014). Development of a fluoride chronic effects benchmark for aquatic life in freshwater. *Environmental Toxicology and Chemistry*, *33*(11), 2621–2627. https://doi.org/10.1002/etc.2724

Mebane, C. A., Chowdhury, M. J., De Schamphelaere, K. A. C., Lofts, S., Paquin, P. R., Santore, R. C., & Wood, C. M. (2020). Metal bioavailability models: Current status, lessons learned, considerations for regulatory use, and the path forward. *Environmental Toxicology and Chemistry*, *39*(1), 60–84. https://doi.org/10.1002/etc.4560

Metcalfe-Smith, J. L., Holtze, K. E., Sirota, G. R., Reid, J. J., & De Solla, S. R. (2003). Toxicity of aqueous and sediment-associated fluoride to freshwater organisms. *Environmental Toxicology and Chemistry*, *22*(1), 161–166. https://doi.org/10.1002/etc.5620220121

Mount, D. R., Erickson, R. J., Highland, T. L., Hockett, J. R., Hoff, D. J., Jenson, C. T., Norberg-King, T. J., Peterson, K. N., Polaske, Z. M., & Wisniewski, S. (2016). The acute toxicity of major ion salts to *Ceriodaphnia dubia*: I. Influence of background water chemistry. *Environmental Toxicology and Chemistry*, *35*(12), 3039–3057. https://doi.org/10.1002/etc.3487

Neuhold, J. M., & Sigler, W. F. (1962). Chlorides affect the toxicity of fluorides to rainbow trout. *Science*, *135*(6), 732–733. https://doi.org/10.1126/science.135.3505.732

Pagenkopf, G. K., Russo, R. C., & Thurston, R. V. (1974). Effect of complexation on toxicity of copper to fishes. *Journal of the Fisheries Research Board Canada*, *31*(4), 462–465. https://doi.org/10.1139/f74-077

Paquin, P. R., Gorsuch, J. W., Apte, S., Batley, G. E., Bowles, K. C., Campbell, P. G. C., Delos, C. G., Di Toro, D. M., Dwyer, R. L., Galvez, F., Gensemer, R. W., Goss, G. G., Hogstrand, C., Janssen, C. R., McGeer, J. C., Naddy, R. B., Playle, R. C., Santore, R. C., Schneider, U.,… Wu, K. B. (2002). The biotic ligand model: A historical overview. *Comparative Biochemistry and Physiology Part C Toxicology and Pharmacology*, *133*(1–2), 3–35. https://doi.org/10.1016/S1532-0456(02)00112-6

Pearcy, K., Elphick, J., & Burnett-Seidel, C. (2015). Toxicity of fluoride to aquatic species and evaluation of toxicity modifying factors. *Environmental Toxicology and Chemistry*, *34*(7), 1642–1648. https://doi.org/10.1002/etc.2963

Pimentel, R., & Bulkley, R. V. (1983). Influence of water hardness on fluoride toxicity to rainbow trout. *Environmental Chemistry*, *2*, 381–386.

Piñeiro, G., Perelman, S., Guerschman, J. P., & Paruelo, J. M. (2008). How to evaluate models: Observed vs. predicted or predicted vs. observed? *Ecological Modelling*, *216*(3–4), 316–322. https://doi.org/10.1016/j.ecolmodel.2008.05.006

Schlekat, C., Stubblefield, W., & Gallagher, K. (2020). State of the science on metal bioavailability modeling: Introduction to the outcome of a society of environmental toxicology and chemistry technical workshop. *Environmental Toxicology and Chemistry*, *39*(1), 42–47. https://doi.org/10.1002/etc.4561

Soucek, D. J., & Dickinson, A. (2016). Influence of chloride on the chronic toxicity of sodium nitrate to *Ceriodaphnia dubia* and *Hyalella azteca*.

Ecotoxicology, *25*(7), 1406–1416. https://doi.org/10.1007/s10646-016-1691-1

Soucek, D. J., & Kennedy, A. J. (2005). Effects of hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. *Environmental Toxicology and Chemistry*, *24*(5), 1204–1210. https://doi.org/10.1897/04-142.1

Soucek, D. J., Linton, T. K., Tarr, C. D., Dickinson, A., Wickramanayake, N., Delos, C. G., & Cruz, L. A. (2011). Influence of water hardness and sulfate on the acute toxicity of chloride to sensitive freshwater invertebrates. *Environmental Toxicology and Chemistry*, *30*(4), 930–938. https://doi.org/10.1002/etc.454

Soucek, D. J., Mount, D. R., Dickinson, A., Hockett, J. R., & Mcewen, A. R. (2015). Contrasting effects of chloride on growth, reproduction, and toxicant sensitivity in two genetically distinct strains of *Hyalella azteca*. *Environmental Toxicology and Chemistry*, *34*(10), 2354–2362. https://doi.org/10.1002/etc.3070

Stephan, C. E., Mount, D. I., Hansen, D. J., Gentile, J. R., Chapman, G. A., & Brungs, W. A. (1985). *Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses*. US Environmental Protection Agency.

Stiff, M. J. (1971). The chemical states of copper in polluted fresh water and a scheme of analysis to differentiate them. *Water Research*, *5*(8), 585–599. https://doi.org/10.1016/0043-1354(71)90114-X

US Environmental Protection Agency. (1980). *Ambient water quality criteria for silver (EPA 440/5-80-071)*. https://www.epa.gov/sites/default/files/2019-03/documents/ambient-wqc-silver-1980.pdf

US Environmental Protection Agency. (1995). *1995 updates: Water quality documents for the protection of aquatic life in ambient water*.

US Environmental Protection. (2016). *Aquatic life ambient water quality criteria cadmium—2016* (EPA-820-R-16-002).

US Environmental Protection. (2018). *Final aquatic life ambient water quality criteria for aluminum* (EPA-822-R-18-001).

Van Genderen, E., Stauber, J. L., Delos, C., Eignor, D., Gensemer, R. W., McGeer, J., Merrington, G., & Whitehouse, P. (2020). Best practices for derivation and application of thresholds for metals using bioavailability-based approaches. *Environmental Toxicology and Chemistry*, *39*(1), 118–130. https://doi.org/10.1002/etc.4559

Varsamos, S., Nebel, C., & Charmantier, G. (2005). Ontogeny of osmoregulation in postembryonic fish: A review [Special issue]. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *141*(4), 401–429. https://doi.org/10.1016/j.cbpb.2005.01.013

Veltman, K., Huijbregts, M. A. J., & Hendriks, A. J. (2010). Integration of biotic ligand models (BLM) and bioaccumulation kinetics into a mechanistic framework for metal uptake in aquatic organisms. *Environmental Science & Technology*, *44*(13), 5022–5028. https://doi.org/10.1021/es903697c

Wang, F., & Song, N. (2021). Modeling of selenite toxicity to wheat root elongation using biotic ligand model: Considering the effects of pH and phosphate anion. *Environmental Pollution*, *272*, 115935. https://doi.org/10.1016/j.envpol.2020.115935

Wright, D. A. (1977). Toxicity of fluoride to brown trout fry. *Environmental Pollution*, *12*, 57–62. https://doi.org/10.1016/0013-9327(77)90008-8

Wurts, W. A., & Perschbacher, P. W. (1994). Effects of bicarbonate alkalinity and calcium on the acute toxicity of copper to juvenile channel catfish (*Ictalurus punctatus*). *Aquaculture*, *125*(1–2), 73–79. https://doi.org/10.1016/0044-8486(94)90284-4

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*(1), 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

