Environmental Toxicology and Chemistry—Volume 43, Number 2—pp. 450–467, 2024
Received: 14 September 2023 | Revised: 31 October 2023 | Accepted: 24 November 2023

Hazard/Risk Assessment

Updated Chronic Copper Bioavailability Models for Invertebrates and Algae

Charlotte Nys,[a,*] Patrick Van Sprang,[a] Stephen Lofts,[b] Stijn Baken,[c] Katrien Delbeke,[d] and Karel De Schamphelaere[e,f]

[a]ARCHE Consulting, Ghent, Belgium
[b]UK Centre for Ecology and Hydrology (UKCEH), Lancaster, UK
[c]International Copper Association, Brussels, Belgium
[d]Consultant, Loonbeek, Belgium
[e]GhEnToxLab, Ghent University, Ghent, Belgium
[f]SYRALUTION, Deinze, Belgium

Abstract: Chronic copper (Cu) bioavailability models have been successfully implemented in European risk assessment frameworks and compliance evaluations. However, they were developed almost two decades ago, which calls for an update. In the study, we present updated chronic Cu bioavailability models for invertebrates and algae. They consider recent ecotoxicity data sets and use the more recent speciation model Windermere Humic Aqueous Model (WHAM) VII and an optimized model structure (i.e., a generalized bioavailability model [gBAM]). Contrary to the classic biotic ligand model, a gBAM models the effect of pH on Cu^{2+} toxicity via a log-linear relationship parametrized through the pH slope S_{pH}. The recalibrated S_{pH} parameters are −0.208 for invertebrates (*Daphnia magna*, two clones) and −0.975 for algae (*Raphidocelis subcapitata* and *Chlorella vulgaris*). The updated models predict 80% to 100% of the observed effect levels for eight different species within a factor of 2. The only exception was one of the two data sets considering subchronic 7-day mortality to *Hyalella azteca*: the prediction performance of the updated invertebrate model at pH ≥ 8.3 was poor because the effect of pH on Cu^{2+} toxicity appeared to be dependent on the pH itself (with a steeper pH slope compared with the updated invertebrate model at pH ≥ 8.1). The prediction performance of the updated Cu bioavailability models was similar to or better than that of the models used for regulatory application in Europe until now, with one exception (i.e., *H. azteca*). Together with the recently published fish bioavailability model, the models developed in the present study constitute a complete, updated, and consistent bioavailability model set. Overall, the updated chronic Cu bioavailability model set is robust and can be used in regulatory applications. The updated bioavailability model set is currently used under the European Union Registration, Evaluation, Authorisation, and Restriction of Chemicals framework regulation to guide the safe use of Cu. *Environ Toxicol Chem* 2024;43:450–467. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Bioavailability; Biotic ligand model; Copper; Aquatic toxicology; Ecological risk assessment

INTRODUCTION

It is well known that chronic copper (Cu) toxicity to aquatic organisms is dependent on the physicochemistry of the surface water. Dissolved organic carbon (DOC), pH, water hardness, and sodium (Na) have been identified as the main toxicity modifying factors for Cu (e.g., Brix et al., 2021; Crémazy et al., 2017; De Schamphelaere & Janssen, 2004, 2006; Nys et al., 2020; Van Regenmortel et al., 2015). During recent years, bioavailability models, such as biotic ligand models (BLMs), have increasingly been used to account for the influence of water chemistry variables in the evaluation of ecological risks of Cu in surface waters. The use of metal bioavailability models to account for bioavailability effects is supported under different European legislation, that is the Registration, Evaluation, Authorisation, and Restriction of Chemicals-framework (REACH; European Chemicals Agency, 2008), the Water Framework Directive (WFD; EU Water Directors, 2018, 2021), and recently also under the Plant

This article includes online-only Supporting Information.

* Address correspondence to charlotte.nys@arche-consulting.be
Published online 29 November 2023 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5796

Protection Product Regulation (PPPR; European Food Safety Authority [EFSA] Plant Protection Regulation-Panel, 2021). Cu bioavailability models were initially implemented to derive predicted no effect concentrations (PNECs) in the risk assessments performed in the European Union under the former Existing Substances Regulation (Cu Voluntary Risk Assessment Report [Cu VRAR]; European Copper Institute, 2008) and more recently also under the REACH directive (European Copper Institute, 2022). In addition, Cu bioavailability models have also been integrated in the European Union WFD to derive Environmental Quality Standards (EQS) because Cu is a river basin-specific pollutant in several member states (EU Water Directors, 2021). User-friendly tools for compliance assessment that rely on Cu bioavailability models are also available (e.g., Peters et al., 2020).

Bioavailability models typically include three components to predict site-specific toxicity: a speciation component, a competition component, and a sensitivity component. The speciation component models the complexation of Cu^{2+} with organic and inorganic ligands because it is assumed that Cu toxicity is primarily related to the activity of the free Cu^{2+} ion, although a few other Cu^{2+} species have been suggested to be bioavailable and contribute to Cu toxicity (e.g., $CuOH^+$ and $CuCO_3$; De Schamphelaere & Janssen, 2004). The competition component incorporates the effects of H^+ and other cations, such as Na^+, Ca^{2+}, and Mg^{2+}, on the uptake of Cu^{2+} into the organism. These effects include—but are not limited to—competition between Cu^{2+} and cations for uptake at the theoretical Cu–biotic ligand site. Finally, the sensitivity component relates the uptake of the free Cu^{2+} ion to the organism's sensitivity. The implementation of bioavailability models into risk assessment procedures requires that, after calibration of the model on the species-specific sensitivity, its performance needs to be investigated for model species using independent data, preferably tested in natural waters (European Chemicals Agency, 2008). In addition, so-called "spot-checking" with nonmodel species also needs to be performed to evaluate the generalization of bioavailability effects within trophic levels (European Chemicals Agency, 2008; EFSA, 2021; EU Water Directors, 2021; hereafter referred to as cross-species validation).

The first chronic Cu bioavailability models were developed two decades ago and were accepted by the European Union and European Union Member States as described in the Voluntary Risk Assessment of copper and copper compounds (European Copper Institute, 2008). That model set included three different freshwaters models, which are hereafter referred to as the original chronic Cu bioavailability models: (1) a model for algae, developed based on *Raphidocelis subcapitata* and *Chlorella vulgaris* (De Schamphelaere & Janssen, 2006), (2) a model for invertebrates, developed based on *Daphnia magna* (De Schamphelaere & Janssen, 2004), and (3) a model for fish, developed by validating an acute *D. magna* model using data for *P. promelas* and *O. mykiss* (De Schamphelaere & Janssen, 2008; Table 1). The original chronic Cu bioavailability models for invertebrates and fish were developed in line with the conventional acute BLM, initially developed by Di Toro et al. (2001). In the original chronic Cu bioavailability models for invertebrates and fish, the effect of pH is modeled via a linear relation between H^+ and the effective concentration expressed as free Me^{2+} (parametrized via the biotic ligand stability constant, K_{HBL}) and the incorporation of biotic ligand binding constants for Cu^{2+} and other relevant Cu species that were assumed to be bioavailable (De Schamphelaere & Janssen, 2004). The bioavailability model for algae demanded a log-linear relationship between Cu^{2+} toxicity and pH (De Schamphelaere & Janssen, 2006; European Copper Institute, 2008). The applicability of the original chronic Cu bioavailability models toward nonmodel species (i.e., cross-species validation) was also demonstrated (De Schamphelaere et al., 2006; European Copper Institute, 2008).

Since the development of the original chronic Cu bioavailability model set, new freshwater chronic toxicity data sets describing bioavailability effects have become available (e.g., Antunes et al., 2012; Crémazy et al., 2017; Van Regenmortel et al., 2015). In addition, new approaches to bioavailability modeling have been developed. For example, a chronic Cu fish BLM-type model has been developed based on a novel toxicity data set for *O. mykiss* (Crémazy et al., 2017). A Cu multiple linear regression (MLR) bioavailability model has been proposed for environmental threshold derivation in the United States (Brix et al., 2021; Mebane, 2023). This model relates dissolved Cu toxicity directly to the toxicity modifying factors (i.e., DOC, hardness, and pH), and therefore does not explicitly consider Cu speciation. Cu generalized bioavailability models (gBAMs) were also developed to describe chronic Cu toxicity to invertebrates (Van Regenmortel et al., 2015) and fish (Nys et al., 2020). The gBAM incorporates the effect of pH on metal toxicity as a log-linear relation between pH and the effective concentration expressed as free metal cation ($ECx_{Me^{2+}}$). While traditional BLMs only consider the competitive effect of H^+ at the biotic ligand site, the log-linear model structure allows gBAMs to consider other factors (e.g., physiological factors) that determine the effect of pH on free metal cation (Me^{2+}) toxicity. In general, the gBAM structure describes the effect of pH on Cu toxicity more accurately compared with the original Cu BLMs (Nys et al., 2020; Van Regenmortel et al., 2015). Generalized bioavailability models are increasingly used in European risk assessment frameworks for different metals, for example nickel (Ni, all trophic levels; Peters et al., 2023) and lead (Pb, algae and fish; Van Sprang et al., 2016).

Geochemical equilibrium speciation modeling of metals, including Cu, in the presence of DOC has also advanced over the past two decades. The Windermere Humic Aqueous Model (WHAM) V is the speciation model underlying all models used in the original chronic Cu bioavailability model set (Table 1). The use of WHAM VII has, over the past decade, gained increased acceptance in the scientific community (Lofts & Tipping, 2011; Tipping et al., 2011). The WHAM VII is the most recent version of the WHAM and incorporates the improved Humic Ion-binding Model VII (Tipping et al., 2011).

Given the progress related to aquatic toxicity data, speciation modeling, and bioavailability model formulations, there is a clear need to update the chronic Cu bioavailability models used in European Risk Assessment Frameworks. Overall, our work aims to update the effects assessment of Cu for the freshwater compartment for use in European risk assessment

TABLE 1: Overview of model parameters of chronic copper (Cu) bioavailability models of the original and the updated chronic Cu bioavailability model set

	Original chronic Cu bioavailability model set[a]			Cu invertebrate BLM refinement	Updated Cu bioavailability model set		
	Invertebrate model	Algae model	Fish model[c]	Invertebrate model	Invertebrate model	Algae model	Fish model
Bioavailability model type[b]	BLM	gBAM	BLM	gBAM	gBAM	gBAM	gBAM
Speciation model	WHAM V	WHAM V	WHAM V	WHAM V	WHAM VII	WHAM VII	WHAM VII
Species used for calibration (see also Supporting Information, S1)	*Daphnia magna* (21-day reproduction)	*Raphidocelis subcapitata; Chlorella vulgaris* (72-h growth rate)	na	*D. magna* (21-day reproduction)	*D. magna* (21-day reproduction)	*R. subcapitata*; *Chlorella vulgaris* (72-h growth rate)	*Oncorhynchus mykiss* (30-day survival)
Independent validation	*D. magna* (21-day reproduction)	*R. subcapitata* (72-h biomass)	na	*D. magna* (21-day reproduction)	*D. magna* (21-day reproduction)	*R. subcapitata* (72-h biomass)	*O. mykiss* (30-day survival, 52-day survival, 52-day ELS weight)
Cross-species validation[e]	*Brachionus calyciflorus* (48-h intrinsic growth rate), *Hyalella azteca* (7-day survival), *Lampsilis siliquoidea* (96-h survival); *Hyridella depressa* (48-h behavior)	*Chlamydomonas reinhardtii* (72-h growth rate)	*O. mykiss* (30-day growth), *Pimephales promelas* (7-day ELS growth)	*B. calyciflorus* (48-h intrinsic growth rate)	*B. calyciflorus* (48-h intrinsic growth rate), *Ceriodaphnia dubia* (7-day reproduction), *Hyalella azteca* (7-day, 10-day-survival), *L. siliquoidea* (96-h survival), *H. depressa* (48-h behavior)	*C. reinhardtii* (72-h growth rate), *Chlorella* sp. (48-h-growth inhibition), *Lemna minor* (7-day- frond number, root length, dry weight)	*P. promelas* (7-day ELS growth, survival, growth rate)
Reference	De Schamphelaere and Janssen (2004); De Schamphelaere et al. (2006); European Copper Institute (2008)	De Schamphelaere and Janssen (2006)	De Schamphelaere and Janssen (2008)	Van Regenmortel et al. (2015), Van Regenmortel (2017)	The present study	The present study	Nys et al. (2020)
Bioavailability model parameters							
S_{pH}	–	−1.354	-	−0.650	−0.208	−0.975	−0.445
Log K_{MgBL}	–	–	3.58	3.53	3.53	–	3.4
Log K_{CaBL}	–	–	3.47	3.53	3.53	–	4.0
Log K_{NaBL}	2.91	–	3.19	2.67	2.67	–	3.0
Log K_{CuBL}	8.02	–	8.02	–	–	–	–
Log K_{CuOHBL}	8.02	–	7.32	–	–	–	–
Log $K_{CuCO3BL}$	7.44	–	7.01	–	–	–	–
Log K_{HBL}	6.67	–	5.40	–	–	–	–
Application range							
pH	5.5–8.5	5.5–8.7	6.0–8.7	5.5–8.6	5.5–8.6	5.5–8.7	5.1–8.7
Ca (mg/L)	2.2–160	2.2–160	na[d]	2.2–160	2.2–160	2.2–160	2.4–124
Mg (mg/L)	0.5–35.4	0.3–24.5	na[d]	0.5–54.0	0.5–54.0	0.3–33.0	1.0–75.4
Na (mg/L)	2.3–506	2.3–485	na	1.6–506	0.5–506	2.3–485	2.5–47.6

[a]The original chronic Cu bioavailability model set represents the bioavailability models that have been used in the Cu Voluntary Risk Assessment Report (European Copper Institute, 2008).
[b]Two types of bioavailability models are represented in the table: (1) biotic ligand model (BLM): a classic linear competition model, (2) generalized bioavailability model (gBAM): a model that integrates a log-linear pH effect that is super-imposed on the linear competition effects of other competing ions besides H^+ (see Equation [1] for model structure).
[c]The fish model selected in the original chronic Cu bioavailability model set was the acute Cu BLM for *D. magna*, which was validated with data of *O. mykiss* and *P. promelas*.
[d]De Schamphelaere and Janssen (2008) reported only a hardness range over which the original chronic Cu fish model has been validated, that is, 30–360 mg $CaCO_3$/L. Note that EU Water Directors (2021) report a range of 3.1–129 mg Ca/L.
[e]In the present study, cross-species validation was defined as a validation of model performance for species that species that have not been used to calibrate the model parameters.
ELS = early life stage; na = not applicable; WHAM = Windermere Humic Aqueous Model.

approaches by (1) incorporating the most recent ecotoxicity data, (2) optimizing the bioavailability model structure, and (3) adopting the most state-of-the-art speciation model. This manuscript describes the update of the chronic Cu algae and invertebrate bioavailability models. The gBAM structure was preferred over other possible model structures because it predicts the effect of pH on Cu toxicity more accurately compared with a traditional BLM (Nys et al., 2020; Van Regenmortel et al., 2015) or an MLR model (Brix et al., 2021). The update was done in three steps. First, Cu bioavailability models for algae and invertebrates were recalibrated in WHAM VII. Second, the models were validated with independent data for model species. Finally, the models were subjected to a 'spot-check' evaluation of the performance for nonmodel species (i.e., cross-species validation). Where possible, the performance of the updated models was compared with the prediction performance of the corresponding original chronic Cu bioavailability models. The updated algae and invertebrate models described in the present study, together with the recent chronic fish bioavailability model (Nys et al., 2020), constitute a complete state-of-the-art validated chronic Cu bioavailability model set covering the three taxa which are the focus of environmental risk assessments.

MATERIALS AND METHODS

For simplicity, in the remainder of this article, the term "updated Cu (bioavailability) model" has been used to refer to a gBAM-type model combined with WHAM VII speciation calculations.

The data used for the development and validation of the updated Cu bioavailability models (also referred to as the "updated models") were those originally used to develop and validate the original chronic Cu bioavailability model set (De Schamphelaere & Janssen, 2004, 2006, 2008, European Copper Institute, 2008; Van Regenmortel et al., 2015), complemented with more recent bioavailability studies. Only chronic bioavailability studies that considered at least two test media with different physicochemistry were retained, with a few exceptions (e.g., mollusc data). Data sets considering only the effects of dissolved organic matter (DOM), while keeping all other physicochemistry parameters constant, were only considered in the Supporting Information because these bioavailability studies do not allow the validation of the competition component of the bioavailability model. A description of the toxicity data used for model calibration and validation and a nonexhaustive overview of excluded data sets is given in Supporting Information, S1. The entire physicochemistry for all bioavailability data sets used for development and validation of the updated models is also provided in Supporting Information, S2.

It should be noted that the scientific name of the green algae *Pseudokirchneriella subcapitata* has recently been updated to *Raphidocelis subcapitata*. In the present study, the most recent name is used to refer to this species (i.e., *R. subcapitata*), while in nearly all referenced studies the former name has been used.

General structure of the Cu generalized bioavailability models

All gBAMs integrate the effect of pH on Me^{2+} toxicity as a log-linear effect between pH and $\log(EC_{Me^{2+}})$; expressed as S_{pH}). This log-linear pH effect is superimposed on the traditional linear effects of the remaining competitive ions at the Cu biotic ligand site, although only when competing cations are of importance for describing Cu toxicity to the considered taxon. The general structure of a Cu gBAM for taxon *y* is given by

$$EC_{Cu^{2+}pred,gBAM,y} = 10^{(Q_{gBAM,y} + S_{pH,gBAM,y} \times pH_i)} \times \left[1 + \sum K_{CatzBL,gBAM,y} \times \{Cat^{n+}_z\}_i\right] \quad (1)$$

In Equation (1), $EC_{Cu^{2+}pred,gBAM,y}$ is the *x*% effective concentration of Cu^{2+}, expressed as free ion activity (mol/L) predicted by the gBAM of taxon *y*, $Q_{gBAM,y}$ is the intrinsic sensitivity of taxon *y* under this gBAM, $S_{pH,gBAM,y}$ is the slope of the log-linear effect of pH on Cu^{2+} toxicity of the chronic Cu gBAM for taxon *y* (unitless), pH_i is the pH of test water *i*, $K_{CatzBL,gBAM,y}$ is the biotic ligand binding constant (in L/mol) in the chronic Cu gBAM for taxon *y* of cation *z*, and $(Cat^{n+}_z)_i$ is the activity (mol/L) of competitive cation *z* in test water *i*. The competition effects of the cations Ca^{2+}, Mg^{2+}, and Na^+ are included in the fish and invertebrate models, but not in the algae model (Table 1).

Speciation assumptions

All speciation calculations were performed using the WHAM VII developed by the Centre of Ecology and Hydrology (CEH; Tipping et al., 2011). The pH buffers 3-(N-morpholino) propanesulfonic acid and 2-(N-morpholino)ethanesulfonic acid were added to the default solute database (pK_a of 7.2 and 6.2, respectively; Kandegedara & Rorabacher, 1999). Default stability constants for inorganic ligand–Cu complexation were adapted to those reported by the National Institute for Standards and Technology (NIST; see Supporting Information, Table S3.1). The default metal–DOM complexation parameters in WHAM VII were used. For modeling the complexation capacity of DOM from natural origin (e.g., field waters), we assumed that 65% of the DOM is reactive and behaves as isolated fulvic acid (FA; 65% active FA). Assumptions of 65% active FA have been shown to typically result in reasonable predictions of metal speciation in natural waters (Ahmed et al., 2014; Tipping, 2002). In addition, it was assumed that DOM contains 50% carbon on a weight basis (Ritchie & Perdue, 2003). Accordingly, the measured DOC concentration (mg/L) was multiplied by 1.3 to obtain the FA concentration (mg/L) to be used as the input for speciation calculations. Furthermore, it was assumed that activities of the metal cation Fe^{3+} are controlled by colloidal $Fe(OH)^3$ precipitates using the default equation and solubility product embedded in WHAM VII (Lofts & Tipping, 2008). The solute and phase databases used for speciation modeling in WHAM VII are available in Supporting Information, S3.

A comparison between the speciation assumption used in the present study (i.e., for WHAM VII speciation calculations) and those used for the original chronic Cu bioavailability models is given in Supporting Information, S3. Van Regenmortel (2017) showed that switching speciation software version of WHAM (from version V to VII) had little effect on model predictions for Cu and Zn bioavailability models.

Development of the updated Cu bioavailability models

The pH slope (S_{pH}; Equation 1) of the invertebrate and algae bioavailability models was specifically calibrated on effect concentrations expressed as free Cu^{2+} activities calculated using WHAM VII ($ECx_{Cu^{2+}}$). The S_{pH} was estimated as the slope of the linear regression between observed log ($ECx_{Cu^{2+}}$) and pH. The S_{pH} was derived based on the average of the slope of the relationship between pH and EC10 and between pH and EC50 for each taxon. This is in line with how the original algae gBAM was developed (De Schamphelaere & Janssen, 2006, European Copper Institute, 2008). However, Van Regenmortel et al. (2015) only considered the EC50 level for deriving the S_{pH} parameter of the WHAM V-based invertebrate gBAM. For the update of the invertebrate bioavailability model, the S_{pH} parameter was calibrated on the toxicity data of two different *D. magna* clones (ARO and K6) tested in synthetic solutions at two pH levels (pH 6.5 and 8.4, four experiments in total; taken from Van Regenmortel et al., 2015). The use of the univariate pH series allows for derivation of an S_{pH} without possible confounding effects of other physicochemical factors.

In the updated invertebrate bioavailability model, the K_{NaBL}, K_{MgBL}, and K_{CaBL} parameters were kept at the same values as in the model developed by Van Regenmortel et al. (2015), that is log $K_{NaBL,invertebrate\ gBAM}$ equal to 2.67 (based on De Schamphelaere & Janssen, 2004) and log $K_{MgBL,invertebrate\ gBAM}$ and $K_{CaBL,invertebrate\ gBAM}$ equal to 3.53 (derived by Van Regenmortel et al., 2015; see Table 1). As such, it was assumed that the switch to a new speciation model only affects the pH slope, and that the cationic biotic ligand constants are not impacted.

Validation of the updated models for model species and nonmodel species

In the validation, the predictive performance of the updated models is evaluated by comparing the observed dissolved Cu toxicity to the predicted dissolved Cu toxicity. Three types of validations were defined in the present study: autovalidation, independent validation, and cross-species validation. An autovalidation is the evaluation of the predictive performance of the model for the data set on which the updated models has been developed, which is an assessment of how well the model is calibrated. An independent validation is the evaluation of the predictive performance of the model for an independent data set, that is, a data set that has not been used to calibrate the updated models. The independent validation data set includes both synthetic and natural waters. Both the autovalidation and the independent validation only consider species on which the model has been originally developed, that is, model species (*D. magna* for the invertebrate model and *R. subcapitata* and *C. vulgaris* for the algae model). A cross-species extrapolation evaluates the prediction performance of the bioavailability model for nonmodel species (i.e., species that have not been used to calibrate the model parameters). The updated invertebrate bioavailability model was used to evaluate prediction performance for all invertebrates, while the updated algae bioavailability model was used for all algae and higher plants. An overview of the data sets that were used for each type of validation is given in Supporting Information, Tables S1.1, S1.2, and S1.3.

The actual bioavailability modeling in the validation process of the updated models consisted of a four-step process (Figure 1). The validation process starts with the calculation of speciation in test media using WHAM VII (Step 1). In Step 2, the intrinsic sensitivity of the updated model for taxon *y* is derived using the following equation, which is derived by rearranging Equation (1):

$$Qx_{gBAM\ taxon,y} = \frac{\sum\left(\log\left(\frac{ECx_{Cu^{2+},observed,i}}{1+\sum K_{Cat_zBL,gBAM,y}\times\{Cat^{n+}{}_z\}_i}\right) - S_{pH,gBAM,y}\times pH_i\right)}{n} \quad (2)$$

where *n* is the number of waters considered to calibrate the intrinsic sensitivity. The intrinsic sensitivity parameter is specific for each species. In addition, the intrinsic sensitivity parameter is calibrated for each effect level, Ecx or no observable effect concentration (NOEC), and for each endpoint separately, because the Cu sensitivity of a species may differ between endpoints. Furthermore, the sensitivity was also calibrated separately for different clones of one species because metal sensitivity can also differ extensively between clones (Messiaen et al., 2013). Although the model can be specifically calibrated for differences in sensitivity, it is assumed that bioavailability relations (e.g., the S_{pH} or the K_{CaBL} value) are similar for different expressions of sensitivity (e.g., different effect levels, different clones, related species). In Step 3, Cu toxicity, expressed as free Cu^{2+} activity, is predicted using the updated Cu bioavailability model (Equation 1) with the intrinsic sensitivity calibrated in Step 2. In Step 4, the predicted free Cu^{2+} toxicity activity is translated back to dissolved Cu toxicity using WHAM VII.

Traditionally, bioavailability model predictions have been considered to be sufficiently accurate if the majority of the effect concentrations were predicted within twofold error (e.g., Di Toro et al., 2001; Garman et al., 2020). In addition, based on the recommendations of Garman et al. (2020), the evaluation of the prediction performance of the bioavailability models also considered the evaluation of prediction bias relative to the considered toxicity modifying factors in the models. In practice, for data sets that evaluate the effect of toxicity modifying factors in a univariate test series, this is done by evaluating the prediction performance of the observed bioavailability trends. For data sets for which physicochemistry parameters have not

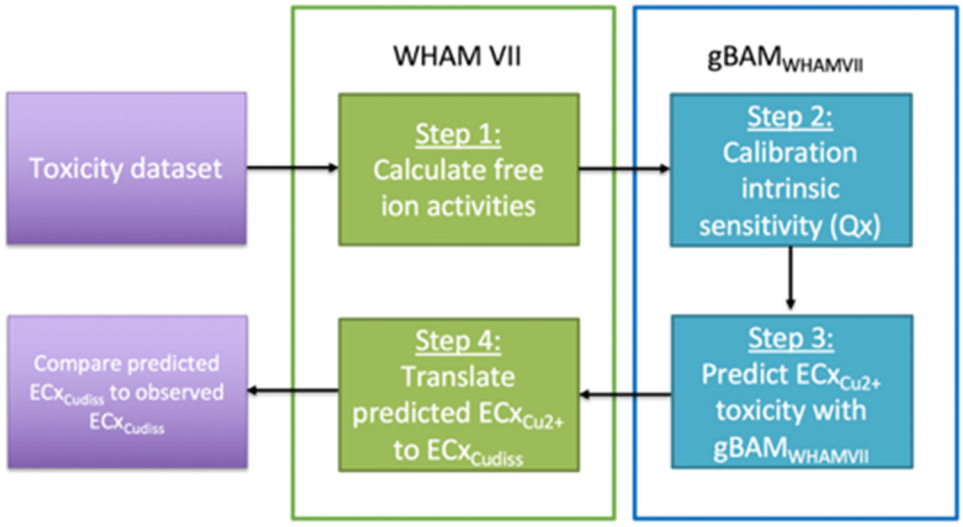

FIGURE 1: Overview of the process used for validating the updated copper (Cu) bioavailability models. The actual bioavailability modeling for the validation process of the updated Cu bioavailability models consists of a four-step process. In Step 1, the Windermere Humic Aqueous Model (WHAM) VII speciation software is used to calculate free Cu^{2+} activity for the validation data set, and depending on the model Mg^{2+}, Na^{+}, and Ca^{2+} activities are also calculated. In Step 2, the intrinsic sensitivity (Qx) is calibrated for the data set under consideration based on WHAM VII calculated speciation generated in Step 1 using Equation 2. In Step 3, Cu toxicity, expressed as free Cu^{2+} activity, is predicted using the updated Cu bioavailability model (Equation 1) with the intrinsic sensitivity calibrated in Step 2. In Step 4, the predicted free Cu^{2+} toxicity activity is translated back to dissolved Cu toxicity using the geochemical speciation software WHAM VII. The observed dissolved Cu toxicity can then be compared with the dissolved Cu toxicity predicted with the updated Cu bioavailability model. EC = effect concentration; gBAM = generalized bioavailability model.

been univariately evaluated, model prediction bias (expressed as predicted/observed Ecx_{diss}) as a function of toxicity modifying factors parameters was further evaluated using the Spearman rank correlation test. Correlations were plotted using the ggpubr package in R. For the evaluation of model prediction bias, preference was given to EC50s (if available) because these are generally estimated with a higher level of precision compared with EC10s (i.e., associated with smaller confidence intervals).

Where possible, the prediction performance of the updated Cu bioavailability models was also compared with that of the original chronic Cu bioavailability model set. This assessment was based on comparing prediction error statistics (mean, median, percentage predicted within twofold error) and cumulative probability plots (Garman et al., 2020).

RESULTS

Calibration and independent validation of the invertebrate model

For the updated invertebrate bioavailability model, the recalibrated S_{pH} parameter represents the average of the log-linear effect of pH on free Cu^{2+} toxicity ($EC50_{Cu^{2+}}$ and $EC10_{Cu^{2+}}$) for the ARO and K6 clones (see Figure 2). The slopes of the two clones and the two considered effect concentrations ranged between −0.049 (ARO clone, $EC50_{Cu^{2+}}$) and −0.392 (K6 clone, $EC50_{Cu^{2+}}$).

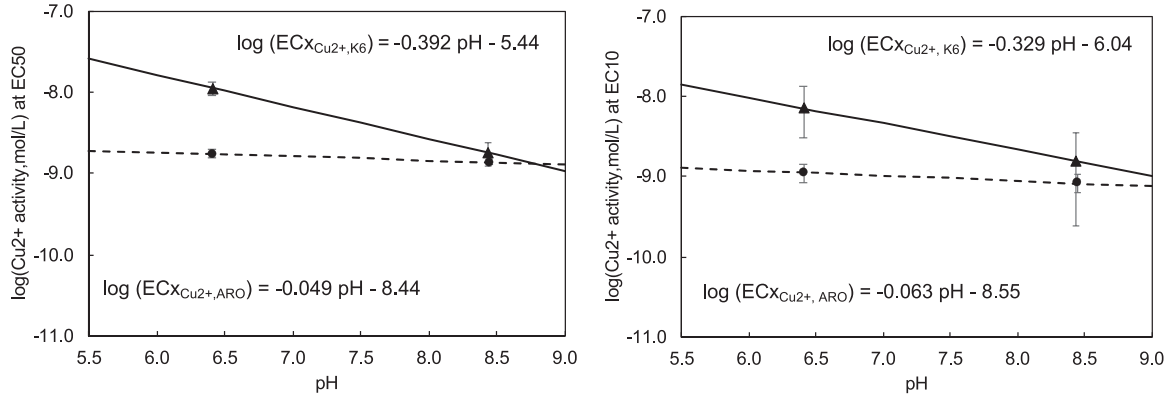

FIGURE 2: Model development: free Cu^{2+} activity (expressed as log_{10}, log [mol/L]) at the 21-day 50% effect concentration (EC50, left panel) and at the 21-day 10% effect concentration (EC10, right panel) as a function of pH for *Daphnia magna*. Symbols represent the K6 clone (triangles) and ARO clone (circles). Free Cu^{2+} activity was calculated using Windermere Humic Aqueous Model (WHAM) VII. Toxicity data was generated by Van Regenmortel et al. (2015). Toxicity for both clones was investigated in natural water at pH 6.4 and 8.4, while keeping all other physicochemistry parameters constant. Error bars denote standard errors on effect concentrations. Linear regression lines and equations are given for both clones.

The recalibrated S_{pH} parameter of the updated model is −0.208 ± 0.089 (average of four slopes ± standard error). An overview of the parameters of the updated chronic Cu invertebrate bioavailability model is given in Table 1. When using these parameters, it was observed that the updated model for invertebrates predicted the data used for their development (i.e., autovalidation) with reasonable accuracy (Supporting Information, S4). The median factor prediction error for the development data sets ranged between 1.1 (ARO clone, $EC50_{Cudiss}$) and 1.2 (K6 clone, $EC50_{Cudiss}$).

Three data sets were available for the independent validation of the updated invertebrate bioavailability model (all considering *D. magna* data; De Schamphelaere & Janssen, 2004; Heijerick et al., 2002; Villavicencio et al., 2011). These include data for two clones obtained both in natural waters ($n = 30$) as well as in reconstituted waters amended with DOM of natural origin ($n = 54$). None of the waters in the independent validation set have been used for the calibration of any of the bioavailability parameters (either S_{pH} or the $K_{Cat,BL}$ parameters) in the invertebrate bioavailability model. For the independent validation, clone-specific intrinsic sensitivities were used to account for possible differences in clone sensitivity. Intrinsic Cu^{2+} sensitivities (*Q*x) for the data sets used for the independent validation of the invertebrate bioavailability model are listed in Supporting Information, Table S5.1. Depending on the selected effect threshold and on the clone, the updated Cu invertebrate bioavailability model predicted the observed effects within twofold error for 82% to 87% of the data points (Table 2 and Figure 3, and Supporting Information, Figure S5.1). Median prediction errors for all waters ranged between 1.2 and 1.5, depending on the clone and the selected effect threshold (Supporting Information, Table S5.2). For natural waters, median prediction errors ranged between 1.3 and 1.8. Overall, predictions of the updated Cu invertebrate bioavailability model for the independent model validation set were deemed very good. Some limited bias was observed in the predictions for one of both clones (K6-clone; Supporting Information, Figures S5.3 and S5.4): the residual variation was significantly correlated with DOC ($p = 0.004$) and Na ($p < 0.001$; mainly driven by high sodium concentrations corresponding to brackish waters).

In general, the performance of the updated invertebrate bioavailability model was more accurate compared with the prediction performance of the original bioavailability model when the same data were considered, despite the lower number of parameters in the updated model (see Supporting Information, Table S7.2 and Figures S7.1a and S7b). Prediction performance was especially improved at low pH (i.e., Skarsjön water with pH = 5.5; Supporting Information, Table S7.3). When compared with the model by Van Regenmortel et al. (2015), the updated invertebrate bioavailability model was only slightly less accurate (Supporting Information, Table S7.2 and Figure S7.1).

Cross-species validation of the updated Cu bioavailability model for invertebrates

Chronic or subchronic bioavailability data sets are available for three nonmodel invertebrate species, *Brachionus calyciflorus*, *Ceriodaphnia dubia*, and *Hyalella Azteca*, from four studies (Supporting Information, Table S1.3; Borgmann et al., 2005; De Schamphelaere et al., 2006; Deaver & Rodgers, 1996; Schwartz & Vigneault, 2006). Intrinsic Cu^{2+} sensitivities (*Q*x) for the different data sets are listed in Supporting Information, Table S8.1. Figure 4 shows the predictive performance of the updated Cu invertebrate bioavailability model for nonmodel invertebrate species. Overall, Cu toxicity to *B. calyciflorus* and *C. dubia* is accurately predicted, with median prediction errors ranging between 1.1 (*B. calyciflorus*) and 1.2 (*C. dubia*; Supporting Information, Table S8.2). For *H. azteca*, the prediction performance was dependent on the considered data set. The 10-day mortality reported by Deaver & Rodgers (1996) was accurately predicted with the updated Cu invertebrate bioavailability model, with median prediction error on median lethal concentration (LC50) equal to 1.2-fold (circles in Figure 4), while 7-day mortality in the data set of Borgmann et al. (2005) was rather poorly predicted (median prediction error on LC50 = 2.2; stripes in Figure 4). The poor prediction performance in the data set of Borgmann et al. (2005) was mainly related to the high pH (pH ≥ 8.1) waters, for which toxicity was consistently underestimated. When the intrinsic sensitivity was calibrated solely on the test media with pH ≤ 7.7, Cu toxicity in test media with pH ≤ 7.7 was accurately predicted, while Cu toxicity in test media with pH ≥ 8.1 was underestimated (Supporting Information, Figure S8.1 and Table S8.3).

Overall, the updated invertebrate bioavailability model predicted the univariate trends of pH and DOC on Cu toxicity to *B. calyciflorus* (Supporting Information, Figure S8.2) and those of pH, Ca (although not at >250 mg Ca/L), Na, and Mg on Cu toxicity to *C. dubia* (Supporting Information, Figure S8.3) reasonably well.

For the natural water data set with *C. dubia* (Schwartz & Vignault 2006), the prediction bias of the updated invertebrate bioavailability model was overall limited and not significantly correlated to the evaluated physicochemistry parameters ($p > 0.05$; Supporting Information, Figure S8.4). For *H. azteca*, there was a clear difference in prediction bias depending on the data set. In the data set of Deaver & Rodgers (1996), the prediction bias was very limited and again not significantly correlated with physicochemistry ($p > 0.05$; Supporting Information, Figure S8.5). For the data set of Borgmann et al. (2005), the residual variation in model predictions is considerably larger (Supporting Information, Figure S8.6). The prediction bias is mainly related to pH ($p < 0.001$), with toxicity at high pH (i.e., pH ≥ 8.3) being consistently underestimated with on average a fivefold difference between observed and predicted toxicity.

In addition to the above described chronic and subchronic data sets, the prediction performance of the updated Cu invertebrate bioavailability model for two molluscs was evaluated based on acute toxicity. Overall, Cu toxicity to early life stages of *Lampsilis siliqouidea* (96-h mortality; data of Wang et al., 2009) and *Hyridella depressa* (48-h valve movement; data of Markich et al., 2003) is relatively well predicted, with median prediction errors ranging between 1.2 (48-h EC50, *H. depressa*) and 1.3 (48-h EC10, *H. depressa* and 96-h LC50, *L. siliquoidea*; Supporting Information, Figure S8.7 and Table S8.4).

TABLE 2: Overview of prediction performance (expressed as percentage predicted within twofold error) of the updated copper (Cu) bioavailability models for data sets considered in the independent validation and the cross-species validation

					% predicted within twofold error[a]		
Bioavailability model evaluated	Species	Reference	Test duration/endpoint(s)/effect level(s)	TMF considered	EC10	EC50/LC50	Other effect level
Updated Cu invertebrate bioavailability model	*Daphnia magna*	De Schamphelaere and Janssen (2004); Heijerick et al. (2002)	21 days/reproduction/K6 clone	DOC, pH, Ca, Mg, Na	85% ($n = 26$)	87% ($n = 45$)	NOEC: 82% ($n = 39$)
		Villavicencio et al. (2011)	21 days/reproduction/ARO clone	DOC, pH, Ca, Mg, Na	–	87% ($n = 39$)	–
	Brachionus calyciflorus	De Schamphelaere et al. (2006)	48 h/intrinsic rate of increase	DOC, pH	100% ($n = 3$)	100% ($n = 3$)	NOEC/LOEC: 100% ($n = 4$)
	Ceriodaphnia dubia	Schwartz & Vigneault (2006)	6–7 days (three broods)/reproduction	DOC, pH, Ca, Mg, Na	–	–	EC25: 92% ($n = 37$)
	Hyalella azteca	Deaver & Rodgers (1996)	10 days/survival	pH, Ca, Mg, Na	–	100% ($n = 5$)	NOEC: 100% ($n = 5$)
		Borgmann et al. (2005)	7 days/survival	pH, Ca, Mg, Na	–	43% ($n = 37$, pH 6.5–8.5) 100% ($n = 23$, pH 6.5–7.7)	
	Lampsilis siliquoidea	Wang et al. (2009)	96 h/survival—early life stage	DOC, pH, Ca, Mg, Na	–	83% ($n = 23$)	–
	Hyridella depressa	Markich et al. (2003)	48 h/valve movement	DOC, pH,	100% ($n = 11$)	100% ($n = 11$)	–
Updated algae bioavailability model	*Raphidocelis subcapitata*	Heijerick et al. (2005)	72 h/biomass yield	DOC, pH, Ca, Mg, Na	89% ($n = 9$)	100% ($n = 8$)	NOEC: 90% ($n = 10$)
	Chlorella sp. (isolate 12)	Wilde et al. (2006)	48 h/growth inhibition	pH	–	80% ($n = 5$)	–
	Chlamydomonas reinhardtii	De Schamphelaere and Janssen (2006)	72 h/growth rate	pH	100% ($n = 3$)	100% ($n = 3$)	–
	Lemna minor	Antunes et al. (2012)	7 days/frond number, net root length, dry weight	DOC, pH, Ca, Mg, Na	–	83%–100% ($n = 6$)	–

[a]Number of datapoints reported between brackets.
DOC = dissolved organic carbon; TMF = toxicity modifying factor.

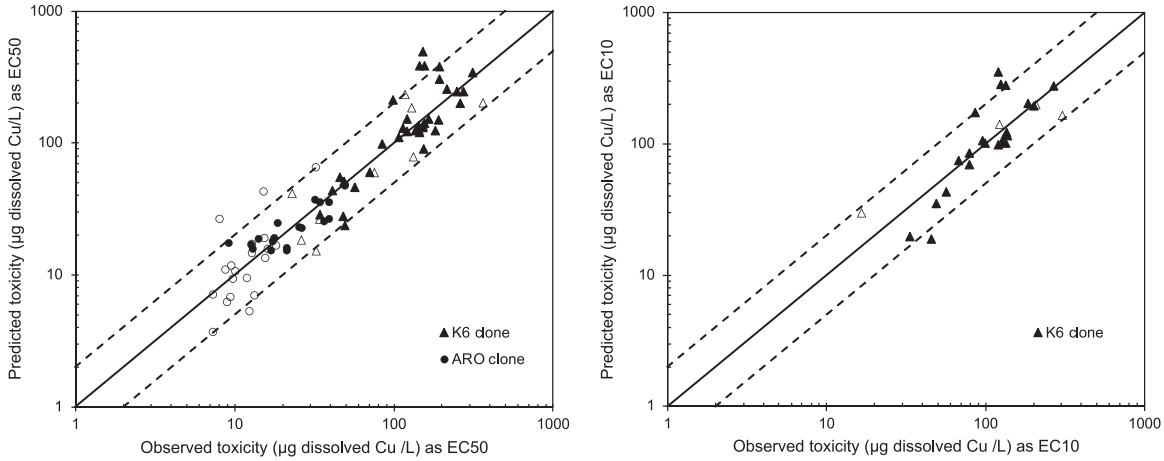

FIGURE 3: Independent validation: prediction capacity of the updated chronic copper (Cu) invertebrate bioavailability model for predicting 21-day toxicity to *Daphnia magna* expressed as 50% effect concentration (EC50, left panel) and 10% effect concentrations (EC10, right panel). The updated chronic Cu invertebrate bioavailability model is a generalized bioavailability model (Equation 1) that is combined with Windermere Humic Aqueous Model (WHAM) VII. Circles represent data points for the ARO clone and triangles represent data points of the K6 clone. Open symbols represent toxicity obtained in test media isolated from natural freshwaters, filled datapoints indicate toxicity obtained in synthetic test water amended with natural organic matter. The dashed lines represent a difference of a factor 2 between predicted and observed toxicity. The solid lines represent a perfect fit between observed and predicted data.

Chronic or subchronic data sets of *B. calyciflorus*, *H. azteca*, and two acute mollusc data sets have previously been used to validate the original chronic Cu bioavailability models. When comparing model predictions of the original invertebrate model (European Copper Institute, 2008) with those of the updated model, model predictions of the updated Cu invertebrate bioavailability model tended to be similar compared with the predictions of the corresponding original chronic Cu bioavailability model (see Supporting Information, Table S7.1), even though the updated model has two parameters less than the original model. The only exception was for the *H. azteca* data set of Borgmann et al. (2005), for which Cu toxicity was predicted with a significantly higher accuracy using the original chronic Cu invertebrate bioavailability model compared with the updated Cu invertebrate bioavailability model, that is 93% and 43% of the datapoints predicted within twofold error, respectively (Supporting Information, Figure S7.3).

Calibration and independent validation of the algae model

The slopes of the log-linear relationship between pH and $ECx_{Cu^{2+}}$ for the two species and the two considered effect concentrations were relatively similar and ranged between

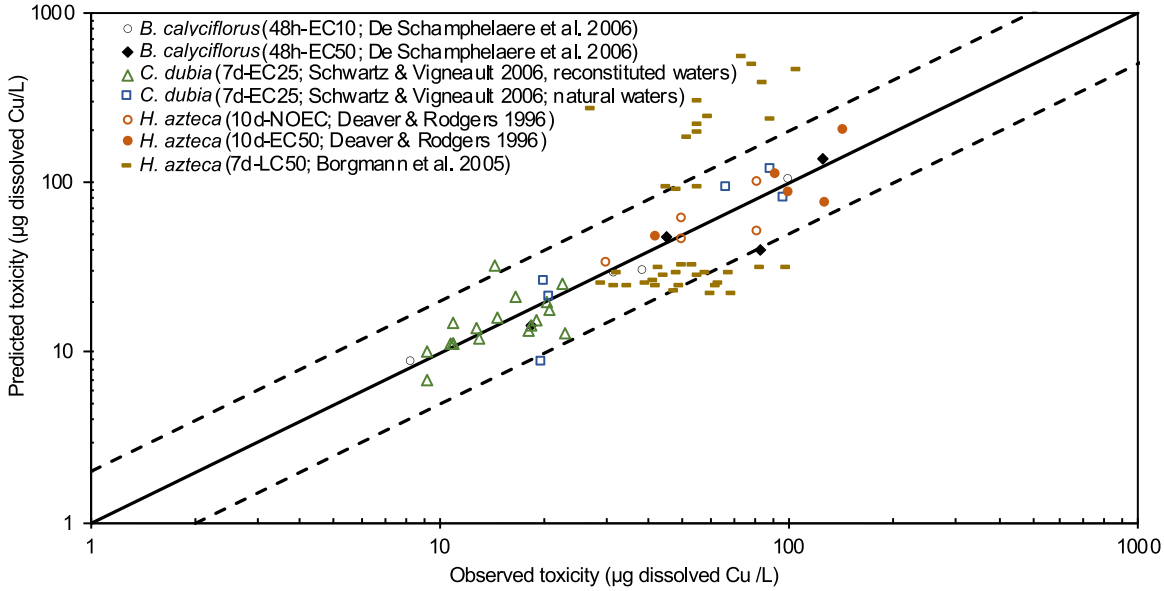

FIGURE 4: Cross-species validation: prediction capacity of the updated chronic copper (Cu) invertebrate bioavailability model for predicting (sub-) chronic toxicity to nonmodel invertebrates. Copper toxicity is expressed as *x*% effect concentration (EC*x*) or no observable effect concentration. The dashed lines represent a difference of a factor 2 between predicted and observed toxicity. The solid line represents a perfect fit between observed and predicted data.

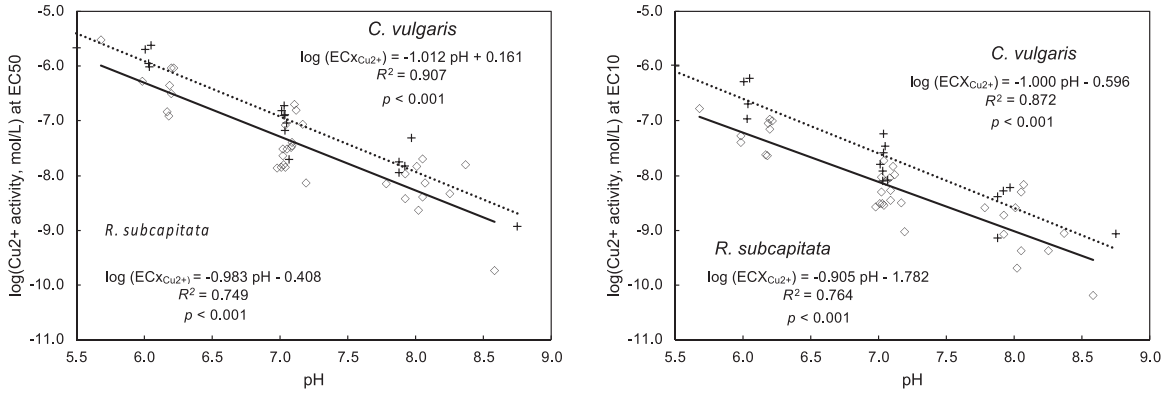

FIGURE 5: Model update: free Cu^{2+} activity (expressed as $\log_{10}$, log [mol/L]) at the 72-h 50% effect concentration (EC50, left panel) and the 72-h 10% effect concentration (EC10, right panel) as a function of pH for *Raphidocelis subcapitata* (diamonds) and *Chlorella vulgaris* (crosses). Free Cu^{2+} activity was calculated using Windermere Humic Aqueous Model VII. Toxicity data were generated by De Schamphelaere & Janssen (2006). Linear regression lines and equations are given for both species.

−0.905 (*R. subcapitata*, $EC10_{Cu^{2+}}$) and −1.012 (*C. vulgaris*, $EC50_{Cu^{2+}}$; Figure 5). The recalibrated $S_{pH,algae\ gBAM}$ was equal to −0.975 ± 0.024 (average of four slopes ± standard error). An overview of the parameters of the updated chronic Cu algae bioavailability model is given in Table 1. When using these parameters, it was observed that the updated model for algae predicted the data used for their development (i.e., autovalidation) with reasonable accuracy (Supporting Information, S4). The median prediction error for the development data sets ranged between 1.2-fold (*C. vulgaris* $EC50_{Cudiss}$) and 1.4-fold (*C. vulgaris* $EC10_{Cudiss}$ and *R. subcapitata* $EC10_{Cudiss}$ and $EC50_{Cudiss}$; Supporting Information, Table S4.4).

For the independent validation of the updated algae bioavailability model one data set is available. Heijerick et al. (2002) reported on Cu toxicity to *R. subcapitata* in a series of natural waters using biomass yield as endpoint. For the independent validation of the updated algae bioavailability model, a data set-specific intrinsic sensitivity was used to account for possible differences in sensitivity between endpoints (growth rate vs. biomass yield; Supporting Information, Table S6.1). The updated Cu algae bioavailability model predicted the $EC10_{Cudiss}$ and $EC50_{Cudiss}$ of *R. subcapitata* for 89% and 100% of the datapoints within twofold error (Figure 6 and Table 2), with median prediction errors ranging between 1.3 ($EC50_{Cudiss}$) and 1.6 ($EC10_{Cudiss}$; Supporting Information, Table S6.2). No bias in the predictions could be identified: the residual variation in the predictions of the updated Cu algae bioavailability model did not show a significant correlation with any of the physicochemistry parameters ($p > 0.05$; Supporting Information, Figure S6.1).

The performance of the updated algae bioavailability model was similar compared with the prediction performance of the original chronic Cu algae model when the same effect level was considered. Both models predicted 90% of the NOECs within twofold error, with an average prediction error of 1.4-fold (see Supporting Information, Table S7.1 and Figures S7.6 and S7.7).

Cross-species validation of the updated Cu bioavailability model for algae

Independent chronic bioavailability data sets are available for three nonmodel algae or plant species: *Chlamydomonas reinhardtii* (De Schamphelaere & Janssen, 2006), *Chlorella* sp. (Wilde et al., 2006), and *Lemna minor* (Antunes et al., 2012; see Supporting Information, Table S1.4). Intrinsic Cu^{2+} sensitivities (*Qx*) for the different data sets are listed in Supporting Information, Table S8.1. Figure 7 shows the

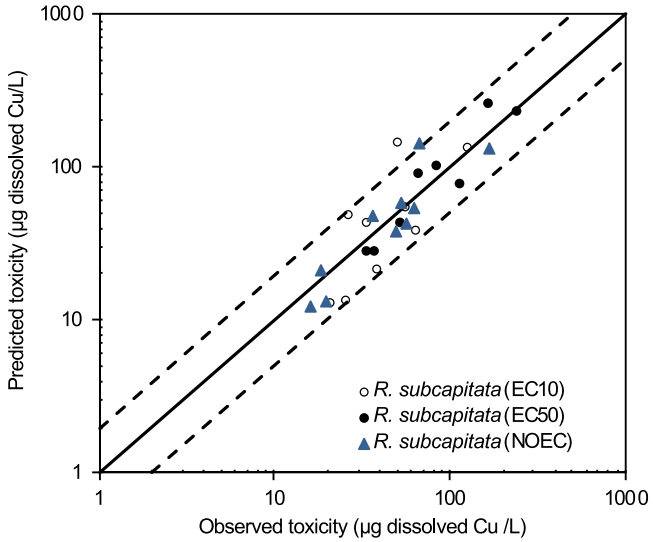

FIGURE 6: Independent validation: prediction capacity of the updated chronic copper (Cu) algae bioavailability model for predicting 72-h toxicity to *Raphidocelis subcapitata* (biomass yield endpoint, data of Heijerick et al. [2002]) as 10% and 50% effect concentration (EC10 [open circles] and EC50 [filled circles], respectively) and no observable effect concentrations (filled triangles). The updated chronic Cu algae bioavailability model is a generalized bioavailability model (Equation 1) that is combined with Windermere Humic Aqueous Model VII. The dashed lines represent a difference of a factor 2 between predicted and observed toxicity. The solid line represents a perfect fit between observed and predicted data.

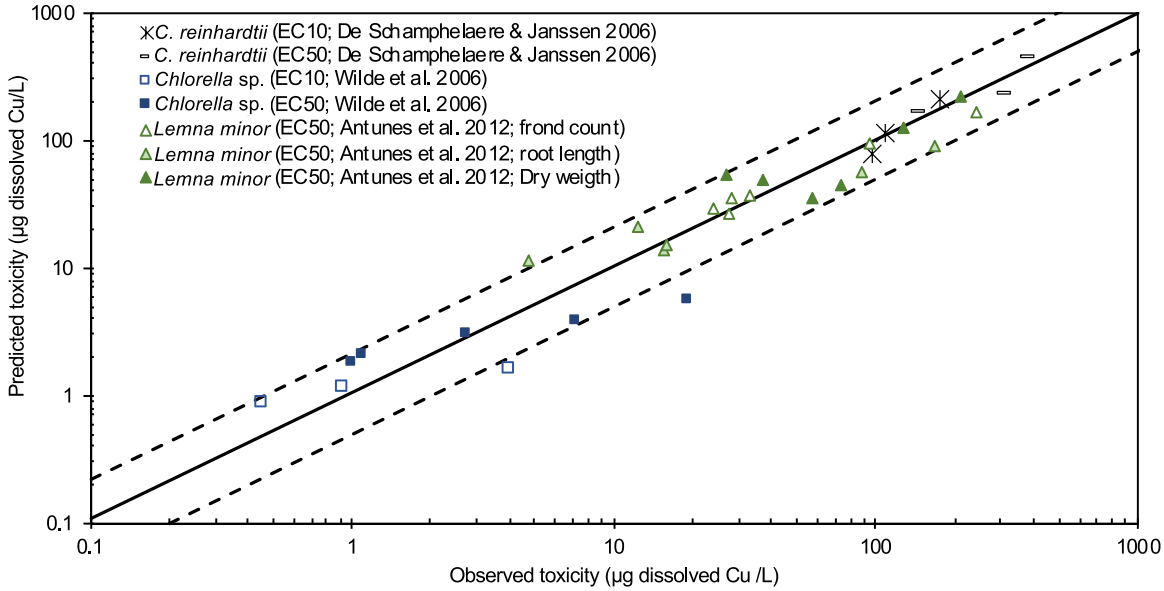

FIGURE 7: Cross-species validation: prediction capacity of the updated chronic copper (Cu) algae bioavailability model for predicting (sub-)chronic toxicity to nonmodel algae and plants. Copper toxicity is expressed as *x*% effect concentration (EC*x*) or no observable effect concentration. The dashed lines represent a difference of a factor 2 between predicted and observed toxicity. The solid line represents a perfect fit between observed and predicted data.

predictive performance of the updated Cu algae bioavailability model for nonmodel algae species. Overall, Cu toxicity to nonmodel algae and plant species was relatively well predicted, with median prediction errors ranging between 1.2 (72-h Cu toxicity to *C. reinhardtii* and 7-day EC50, *L. minor*-frond count) and 1.8 (48-h Cu toxicity to *Chlorella* sp.; Supporting Information, Table S8.5). In addition, 80% to 100% of the toxicity datapoints were predicted within twofold error (Table 2), depending on the species and endpoint. The updated algae bioavailability model predicted the observed trend of increasing dissolved Cu toxicity with increasing pH for *C. reinhardtii* almost perfectly (Supporting Information, Figure S8.8, left panel). The predicted trend of increasing Cu toxicity with increasing pH for the *Chlorella* sp. data set was also reflected in the observed toxicity data. However, the observed trend for *Chlorella* sp. seemed to be steeper than predicted by the updated algae bioavailability model (Supporting Information, Figure S8.8, right panel). The latter implicates that a higher S_{pH} might be optimal for this species. The corresponding S_{pH} for this species would be −1.463 (Supporting Information, Figure S8.10). For *L. minor*, the residual variation in model predictions is overall limited (Supporting Information, Figure S8.9). A small prediction bias was tentatively observed related to pH, although this was not statistically significant ($p = 0.14$).

The toxicity data sets of *C. reinhardtii* have previously been used to validate the original chronic Cu algae bioavailability model. When comparing model predictions of the original chronic Cu algae model with those of the updated algae model, model predictions of the updated Cu algae bioavailability model tended to be similar compared with the predictions of the corresponding original chronic Cu bioavailability model (see Supporting Information, Table S7.1 and Figure S7.6).

DISCUSSION

Update of the Cu bioavailability models—Speciation and competition component

In the present study, we updated the existing chronic Cu bioavailability models for invertebrates and algae used in European risk assessment frameworks and EQS derivation based on the most recent available science. This update consisted of (1) a recalibration of the bioavailability models using the state-of-the-art speciation model (WHAM VII; Tipping et al., 2011) and (2) a revision of the invertebrate bioavailability model structure (i.e., gBAM-model structure; Van Regenmortel et al., 2015).

The use of the WHAM VII (updated models) versus WHAM V (original models) for modeling Cu^{2+} in the speciation component of the bioavailability model includes a switch to the Humic Ion-binding Model VII. Model VII replaces the previous versions of the Humic Ion-binding Model (Models V and VI). Compared with Model V, Model VII takes better account of binding site heterogeneity and allows a better description of metal binding at higher pH compared with older versions (Tipping et al., 2011). The model computes metal interactions with natural organic matter based on binding to strong acid (carboxylate) and weak acid (phenolic) functionalities, including multidentate binding, and uses an additional term to account for the presence of particularly strong multidentate sites, which likely involve amino and/or thio-based functional groups. WHAM VII has been shown to be able to reproduce field measurements of free metal ion concentrations relatively accurately for several metals, such as

Cu, at concentrations relevant for metal toxicity (Ahmed et al., 2013, 2014; Lofts & Tipping, 2011). In addition to the change in speciation model, assumptions of the speciation component of the Cu bioavailability models were modified in terms of percentage active FA in DOM (% active FA), inclusion of competition between Fe^{3+} and Cu^{2+} for binding to DOM (based on Lofts & Tipping, 2011), and small updates in NIST stability constants for CuOH and $CuCO_3$ (see Supporting Information, Table S2.1). Within the speciation component, the percentage of active FA has been set to 65% based on, for example, Ahmed et al. (2014). These authors showed that this assumption resulted in reasonable predictions of Cu^{2+} speciation in polluted and unpolluted natural waters. However, it should be noted that the percentage of active FA is typically dependent on the DOM source of the receiving water (see e.g., De Schamphelaere et al., 2003). Techniques to measure or approximate the percentage of active FA certainly exist (e.g., Ahmed et al., 2014; De Schamphelaere et al., 2003), and De Schamphelaere & Janssen (2006), for example, have implemented source-specific percentages of active FA in their speciation component underlying the original chronic Cu invertebrate bioavailability model. It is expected that this adaptation may improve model fits. Indeed, De Schamphelaere & Janssen (2006) noted that when the source-specific the percentage of active FA was used, bioavailability model predictions improved compared with when a default percentage of active FA was used (i.e., 50% active FA), especially in the low-concentration range. However, measurements of active FA percentages or surrogates thereof, such as UV absorbance, are not routinely implemented in compliance evaluation programs in Europe. As such, a pragmatic choice was made to apply the 65% active FA assumption for all DOM sources across the data sets considered in the present study.

In the present study, the competition component of the chronic Cu invertebrate and algae bioavailability models have been updated to a WHAM VII-based gBAM structure. For this update, the S_{pH} parameter, expressing the effect of pH on log (Cu^{2+}) toxicity, was recalibrated using the same data sets as used for calibration of the S_{pH} parameter in the original models, but using WHAM VII as speciation model instead of WHAM V. Recalibrated S_{pH} values were equal to −0.208 for *D. magna* and −0.975 for algae (based on toxicity data for two species: *R. subcapitata* and *C. vulgaris*). These values are lower than their WHAM V counterparts, that is −1.354 for algae (De Schamphelaere & Janssen, 2006) and −0.650 for *D. magna* (Van Regenmortel et al., 2015). These lower S_{pH} values most likely are the result of the different approach of modeling of metal binding to DOM at higher pH in WHAM VII. Especially at high pH a large difference in modeled free Cu^{2+} activity was noted between our calculations executed in WHAM VII and the WHAM V-based calculations of Van Regenmortel et al. (2015). Overall, at high pH, WHAM VII predicts lower complexation of Cu on the DOC binding sites compared with WHAM V. As such, a higher free Cu^{2+} activity at EC50 in the *D. magna* univariate pH series is predicted at high pH in WHAM VII (i.e., almost one order of magnitude higher free Cu^{2+} activity in WHAM VII compared with WHAM V; Supporting Information, Figure S3.1), which is also reflected in the lower S_{pH} value.

Recently, Nys et al. (2020) also updated the Cu fish bioavailability model to a WHAM VII-based gBAM structure model. The recalibrated S_{pH} for juvenile rainbow trout in the model developed by these authors was equal to −0.445 (Table 1). Overall, this range in S_{pH} for the different trophic levels shows that effect of pH on log (Cu^{2+}) toxicity is stronger for algae/plants compared with animal species. However, the S_{pH} can also differ between species (Supporting Information, Figure S8.10) and endpoints (Supporting Information, Figure S8.11), and even between clones (as visualized for the K6 clone and ARO clone of *Daphnia magna* in Figure 1). For example, the S_{pH} for different algae and plants variates between −0.965 (*C. reinhardtii*) and −1.471 (*Chlorella* sp.; Supporting Information, Figure S8.10).

Due to its log-linear model structure, a gBAM may in a nonmechanistically manner account for other factors (e.g., physiological factors) that determine the effect of pH on bivalent metal cation (Me^{2+}) toxicity besides the competitive effect of H^+ at the biotic ligand site. These additional mechanisms can include the contribution of Cu complexes (e.g., $CuOH^+$ and $CuCO_3$) to Cu toxicity (Erickson et al., 1996; Grosell et al., 2004), the effect of pH on membrane permeability (Lavoie et al., 2012) and/or differences between bulk pH and the pH of the microenvironment surrounding the Cu uptake sites (e.g., Playle & Wood, 1989; Tao et al., 2002). By using a gBAM-type model, fewer model parameters are typically needed compared with the classic linear BLM. For instance, three parameters, that is K_{HBL}, K_{CuOHBL}, and $K_{CuCO3BL}$, of the original chronic Cu invertebrate BLM of De Schamphelaere & Janssen (2004) were replaced by one single parameter, S_{pH}, in the updated model for this species. Similarly, Nys et al. (2020) also reported that the gBAM-type model greatly reduced the complexity of the Cu fish bioavailability model compared with previous BLM-type models (fish models of Crémazy et al., 2017; De Schamphelaere & Janssen, 2008).

One could question the general applicability of the updated invertebrate model given that the S_{pH} parameter of the model was derived on only two datapoints per clone (four datapoints in total) and the large difference in derived S_{pH} slope observed between both *D. magna* clones. As such, the effect of pH on free Cu^{2+} toxicity to *D. magna* across both clones was further evaluated by considering all available toxicity data obtained in synthetic test media (Figure 8). When a broader set of datapoints is considered, the corresponding pH slopes (S_{pH}) for both clones ranged between −0.131 (ARO clone, 24 data points, pH 6.3–8.5) and −0.380 (K6 clone, 40 data points, pH 6.1–8.44). Overall, this analysis shows again the difference in pH slope between both clones. Averaged over both clones the S_{pH} is equal to −0.256, which is close to S_{pH} of the updated Cu invertebrate model ($S_{pH} = -0.208$).

While the original chronic Cu invertebrate model did not consider the competition effects of Ca and Mg, the updated chronic Cu invertebrate model includes biotic ligand binding constants for both Ca and Mg. The consideration of hardness competition effects in the updated invertebrate model followed

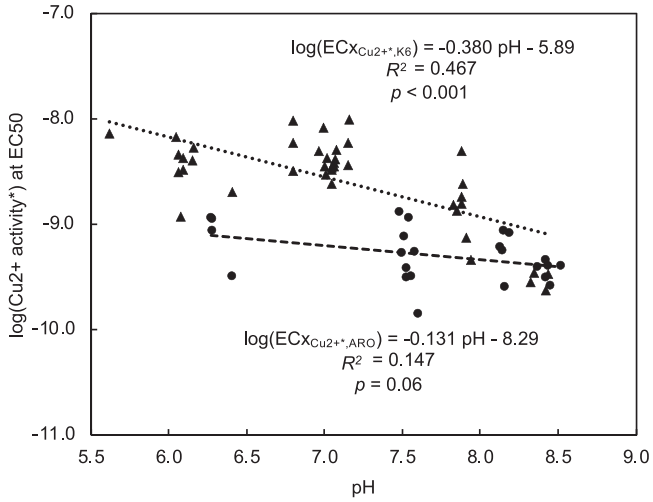

FIGURE 8: Free Cu^{2+} ion activity at the 21-day 50% effect concentration (EC50) as a function of pH for the K6 (triangles) and ARO (circles) *Daphnia magna* clones. Only toxicity data obtained in synthetic test media have been included. The asterisks indicate that activities have been corrected for sodium, calcium, and magnesium effects (Van Regenmortel 2017). Chronic toxicity data originated from De Schamphelaere & Janssen (2004), Heijerick et al. (2002), Villavicencio et al. (2011), and Rodriguez & Arbildua (2012).

Van Regenmortel et al. (2015), who considered the hardness competition effect for *D. magna* previously observed by Rodriguez & Arbildua (2012). Van Regenmortel et al. (2015) reported that incorporating a hardness competition effect in their gBAM-type model improved model predictions at low hardness compared with alternative gBAM model types without Ca and Mg competition effects.

In the updated Cu invertebrate bioavailability model, the values of the cationic biotic ligand binding constants (i.e., K_{NaBL}, K_{MgBL}, and K_{CaBL}) were retained from the existing chronic Cu bioavailability model for invertebrates (i.e., the gBAM-model from Van Regenmortel et al., 2015). As such, it was assumed that the switch to a new speciation model only affects the pH slope and that the cationic biotic ligand constants are not impacted. The validity of this assumption was assessed by fitting the K_{NaBL}, K_{CaBL}, and K_{MgBL} parameters simultaneously with the S_{pH} parameter. This was done using a Markov Chain Monte Carlo (MCMC) algorithm on WHAM VII-based speciation, which was applied on all synthetic test media in the *D. magna* data set (see methodology in Supporting Information, S9). In addition, clonal differences (K6 clone vs. ARO clone) in cationic biotic ligand binding constant estimates and in Cu sensitivity were evaluated by implementing separate parameter optimalization runs for both clones. Overall, the optimal parameter estimates resulting from the MCMC run were relatively close to the values retained in the updated invertebrate model, that is, a maximum 1.3-fold difference in parameter values was observed (Supporting Information, Table S9.1). Overall, there was limited difference between both clones in MCMC optimal parameter estimates, that is, maximum 1.2-fold difference in MCMC-optimized biotic ligand binding constants. In conclusion, the assumption taken for the development of the updated chronic Cu invertebrate bioavailability model that the K_{NaBL}, K_{MgBL}, and K_{CaBL} parameters are not influenced to a large extent by the switch of speciation software version is valid. In addition, it suggests that cationic competition effects for Ca, Mg, and Na are in reasonable agreement between different clones of *D. magna*. The optimal S_{pH} parameter for both clones resulting from the MCMC fitting (−0.219 for K6 and −0.203 for ARO) was also very close to the one of the updated chronic Cu invertebrate bioavailability model (−0.208). Overall, this confirms the robustness of the updated invertebrate model.

Overall, there is nonnegligible variability in the slope (S_{pH}) of the log-linear relationship between pH and Cu^{2+} toxicity between different species, even when these species are relatively closely related, for example, for green algae the S_{pH} ranges between −0.965 and −1.471, or even for clones of the same species (although these appeared to be more similar when a broader data set was considered). This variation in pH slopes may in future updates of the Cu bioavailability models be addressed with a probabilistic approach of sampling S_{pH} values for uninvestigated species from a statistical distribution fitted to reported S_{pH} values for other species, compared with the current approach that selects a single fixed slope for an entire trophic level. Additional work may also consider optimizing the model structure that accounts for the competition effects of Ca^{2+}, Mg^{2+}, and Na^{+}, for instance by also expressing these too as log-linear effects rather than the current approach, which considers these as linear competition effects resulting from the assumption of competition by all these cations with copper for a single biotic ligand site.

Validation for model species and nonmodel species

The present study showed that the updated chronic Cu invertebrate and algae bioavailability model predicted the observed toxicity from independent data sets (i.e., data sets not used for model development) accurately, and for model species as well as for most nonmodel species. A bioavailability model is generally considered sufficiently accurate if the majority of ECx_{Mediss} of an independent data set are predicted within twofold error (Di Toro et al., 2001; Garman et al., 2020). This twofold error range reflects the expected random variation in toxicity test results. In total, data for eight species originating from 11 different (sub-)chronic studies were considered in the independent validation (model species *D. magna* and *R. subcapitata*) and the cross-species validation (nonmodel species *B. calyciflorus*, *C. dubia*, *H. azteca*, *Chlorella* sp., *C. reinhardtii*, and *L. minor*) of the updated model for algae and invertebrates. Across all data sets, dissolved Cu toxicity was predicted within twofold error for at least 80% of the considered datapoints (Table 2), with one exception (i.e., the 7-day mortality *H. azteca*; data set of Borgmann et al., 2005).

Other considerations in bioavailability model performance evaluations, such as model prediction bias in relation to physicochemistry parameters, have recently received increasing

attention (Garman et al., 2020). The updated models demonstrate no or limited prediction bias when applied to 10 data sets covering eight species. The exception is *H. azteca*, for which a clear bias toward pH was observed in one of both data sets (Borgmann et al., 2005, see next paragraph). Based on this evidence, it is concluded that the updated chronic Cu invertebrate bioavailability model can be extrapolated to other cladocerans (based on *C. dubia*) and rotifers (based on *B. calyciflorus*), while the updated Cu algae bioavailability model can be extrapolated to algae (based on *Chlorella* sp. [tropical isolate] and *C. reinhardtii*) and higher plants (based on *L. minor*).

For amphipods *(H. azteca*), the analysis of the applicability of the updated invertebrate bioavailability model to predict Cu toxicity to amphipods was ambiguous. For one data set (Deaver & Rodgers, 1996), the updated invertebrate bioavailability model resulted in good predictions of Cu toxicity, that is, all LC50s were predicted within twofold error. However, this data set is accompanied by some uncertainties because the physicochemistry was estimated based on pH, hardness, and alkalinity and DOC was estimated based on default assumptions. On the other hand, for the data set of Borgmann et al. (2005), which is more extensive and is based on measured physicochemistry, considerable bias in model predictions was observed. This bias was mainly related to pH. At pH ≤ 7.7, toxicity tended to be overestimated by the updated invertebrate bioavailability model (i.e., relative prediction error <1.0), while at pH ≥ 8.1 toxicity tended to be underestimated up to fivefold. However, recalibrating the model on only those test media with pH ≤ 7.7 resulted in accurate predictions for all test media with pH ≤ 7.7, while at pH ≥ 8.1 toxicity remained underestimated (Supporting Information, Figure S8.1). Overall, this shows that the updated chronic invertebrate model can be used for predicting Cu toxicity to *H. azteca* with reasonable accuracy up to pH 7.7. The pH-related bias in model predictions can be explained by the fact that the observed pH slope in the *H. azteca* data set of Borgmann et al. (2005) is steeper (i.e., $S_{pH} = -1.117$, considering all pH levels) compared with that of the updated invertebrate bioavailability model (i.e., $S_{pH} = -0.205$; Supporting Information, Figure S8.11). However, the data also suggests that the relationship between pH and Cu^{2+} toxicity may not be log-linear. When fitting a log-linear relationship, the S_{pH} is much steeper at pH above 8.1 ($S_{pH} = -1.671$) compared with that at pH below 8.0 ($S_{pH} = -0.442$). A similar dependency of the S_{pH} parameter on the considered pH level has previously been reported for Ni^{2+} toxicity for five different aquatic organisms (Nys et al., 2016). This pH dependency of the S_{pH} has been integrated in the chronic Ni bioavailability models for algae and invertebrates (both gBAM-type models) via a two-step normalization procedure, where different pH slopes are applied dependent on the considered pH level in the target water (Nys et al., 2016; Peters et al., 2023). A similar procedure could be considered for predicting Cu toxicity to *H. azteca*. Borgmann et al. (2005) have already explored other options for bioavailability modeling for this data set because they observed that toxicity was not consistent with a single-binding-site BLM. As such, they developed a two-binding-site model, with separate coefficients for the effects of Ca^{2+} and Na^{+} at low and high pH. Borgmann et al. (2005) showed that this more complex two-binding-site model could indeed accurately predict 7-day Cu toxicity to *H. Azteca*, although this may not be surprising given the higher number of model parameters. Recently, Mebane (2023) also investigated the data set of Borgmann et al. (2005) for the evaluation of the predictive performance of a Cu MLR model. Mebane (2023) reported that the *H. azteca* data set was unusable to evaluate bioavailability model performance, which the author related to uncertainties in DOC values resulting from the static, nonrenewal exposures with food addition. Borgmann et al. (2005) reported that DOC concentrations during the exposure increased from ≦0.2 mg/L in the artificial test media and 1.1 mg/L for tap waters prior to test initiation to 0.4 to 2.8 mg/L (with an average of 1.7 mg/L) depending on the test treatment. Mebane (2023) noted that these uncertainties could lead in a factor 10-difference in predictions in some conditions (notably low alkalinity). Given the above discussion, there is a clear need to further investigate bioavailability relations for *H. azteca*. Moreover, because presently only subchronic (i.e., 7 or 10 days) bioavailability data sets are available for *H. azteca*, it is recommended to evaluate these bioavailability relationships during chronic exposures, that is, 14 or 28 days as defined in the International Organization for Standardization (ISO) protocol (ISO, 2013) or even 42 days as recently proposed (Ivey et al., 2016). In addition, it would also be useful to evaluate the cross-species extrapolation of the updated chronic Cu model to a broader set of invertebrate species, and to evaluate the variation of the dependency of Cu toxicity in relation to toxicity-modifying factors across species.

For molluscs, a data set is available evaluating the effect of DOC on chronic 28-day Cu toxicity to *Villosa iris* (Wang et al., 2011). This data set only allows evaluation of the speciation component of the updated invertebrate bioavailability model for molluscs, and does not allow evaluation of the competition component because all other toxicity-modifying factors for Cu (i.e., pH, Ca, Mg, and/or Na) were kept constant. Overall, the effect of DOC on chronic Cu toxicity to *V. iris* could be predicted, although only when uncertainties related to measurement of low DOC concentrations were accounted for (see Supporting Information, Figure S10.3). Given the shortage of chronic true bioavailability data sets (i.e., considering other parameters than DOC) for molluscs, two data sets reporting on short-term Cu toxicity with sensitive life stages (i.e., juvenile *L. siliquoidea*, 96-h mortality; Wang et al., 2009) and for relative sensitive endpoints (48-h valve movement for *H. depressa*; Markich et al., 2003) were considered. The updated Cu invertebrate bioavailability model predicted short-term Cu toxicity on survival of *L. siliquoidea* juveniles and on valve movement of *H. depressa* for at least 83% of the data points within twofold error (Table 2). Although these mollusc data represent short-term test duration, the sensitivity of these species' life stages and endpoints are within the range of chronic Cu sensitivity of the six mollusc species for which data is available in the most recent chronic Cu freshwater database used for PNEC derivation under REACH (European Copper

Institute, 2022). When normalized to the most sensitive ecoregion, Swedish Lake (pH 6.7, DOC 3.8 mg/L, hardness 28 mg $CaCO_3$/L), the acute LC50 of *L. siliquoidea* (22.2 µg Cu/L) falls within the range of normalized chronic EC10 or NOECs for molluscs that are used for PNEC derivation normalized to the same conditions (i.e., ranging between 5.8 µg Cu/L [*Vilosa iris*, growth] and 38.6 µg Cu/L [*Lymnaea stagnalis*, reproduction]; Supporting Information, Table S1.4). For *H. depressa*, the normalized 48 h EC10 is only threefold higher (i.e., 104.9 µg Cu/L) when compared with the range of chronic EC10s for molluscs normalized to the same conditions. Overall, the comparison of normalized acute versus chronic sensitivity for molluscs is in line with Wang et al. (2007), who reported that the acute to chronic ratio for *L. siliqouidea* is relatively small (i.e., 4) for Cu. Therefore, the available data suggests that the updated chronic Cu invertebrate bioavailability model can be extrapolated to predict chronic toxicity to molluscs, although there remains some uncertainty. The relationship between water chemistry and Cu toxicity may vary depending on the exposure period, as evidenced by the different model parameters for *D. magna* in the acute BLM (De Schamphelaere & Janssen, 2002) and chronic BLM (De Schamphelaere & Janssen, 2004). Additional experimental evidence on the effect of varying water chemistry on chronic Cu toxicity to molluscs would give a definitive conclusion on the applicability of the updated chronic Cu invertebrate bioavailability model for molluscs during prolonged exposures.

For those data sets that have previously been used to validate the original models, we compared model predictions of the original chronic Cu models with those of the updated models (see Supporting Information, Table S7.1). The updated Cu invertebrate model is more accurate for *D. magna*. This is because the updated model recognizes the differences in the effect of pH on Cu^{2+} toxicity across laboratories and clones of *D. magna*, while the original model considered only one clone (K6 clone). For most other species (i.e., *B. calyciflorus*, *L. siliquoidea*, *H. depressa*, *R. subcapitata*, and *C. reinhardtii*), predictions by the updated models were very similar compared with the predictions of the corresponding original model, in spite of the lower number of model parameters in the updated invertebrate model. The only exception is the data set of *H. azteca* of Borgmann et al. (2005), which was predicted with a significantly higher accuracy using the original chronic Cu invertebrate model compared with the updated invertebrate model. The latter may suggest that the BLM structure of the original invertebrate Cu bioavailability model is able to capture the bioavailability relationships more accurately in this data set compared with the current updated invertebrate bioavailability model. Alternatively, a gBAM-type model with a higher pH slope (i.e., S_{pH}) and/or separate pH slopes dependent on the considered pH could also have the potential to predict toxicity to *H. azteca* more accurately compared with the updated invertebrate bioavailability model (as discussed above).

Because bioavailability models are calibrated and validated within a defined range of physicochemistry conditions, the application of these models within these ranges ensures the reliability of regulatory assessments (European Chemicals Agency, 2008; EU Water Directors, 2021). Application of bioavailability models outside these ranges increases uncertainty regarding bioavailability relationships. The application range of a bioavailability model can be defined as the combined physicochemistry range over which a bioavailability model has been calibrated or validated (EU Water Directors, 2021). The application ranges of the updated models for invertebrates and algae are largely similar to those of the original models (Table 1). Considering the application ranges of the three models in the updated bioavailability model set, it can be concluded that the combined application range of the updated model set is between a pH of 5.5 and 8.6, between Ca concentrations of 2.4 and 124 mg/L, between Mg concentrations of 1.0 and 33 mg/L, and between Na concentrations of 2.5 and 47.6 mg/L. The pH range is broader than the application range of the original chronic Cu bioavailability model set, while the Ca range is relatively similar (pH 6.0–8.5, Ca 3.1–129 mg/L).

To be able to use the updated models to predict Cu sensitivity in local freshwaters, the most critical input parameters for the model equations are pH, DOC, Ca Mg, and Na. In addition, the associated speciation calculations require a more extensive set of input parameters and also need to consider K, Cl, SO_4, alkalinity, and temperature. Bioavailability models are typically data-hungry in terms of the needed physicochemistry data, while not all these parameters are routinely measured in monitoring frameworks. However, it has previously been suggested that for Mg, Na, Cl, SO_4, and alkalinity, if measurements are not available, estimates from standard regression, log-linear relationships between these parameters and Ca in European waters can be used, for instance those that have been published by Peters et al. (2011). However, it should be noted that using estimates rather than measurements adds uncertainties to bioavailability model calculations.

Relevance of the updated Cu bioavailability model set for risk assessment in Europe

The updated models developed in this manuscript, together with the recently published chronic Cu fish bioavailability model (Nys et al., 2020), constitute a complete, consistent, and updated Cu bioavailability model set. It uses the most recent version of the WHAM VII, and all models are of the gBAM type, which has an improved estimation of the effect of pH on Cu toxicity compared with the classic BLM-type model (Nys et al., 2020; Van Regenmortel et al., 2015). The gBAM structure has the advantage that it avoids the need to consider the potential toxicity of CuOH and $CuCO_3$, thereby reducing the number of model parameters without losing predictive power. In addition, the independent validation and cross-species validation of the updated bioavailability models considered a more extensive data set compared with those used for the original chronic Cu bioavailability model set, both recognizing additional data sets (e.g., Antunes et al., 2012; Schwartz & Vigneault, 2006; Villavicencio et al., 2011; Wilde et al., 2006) as well as additional effect levels more relevant in current risk assessment applications (e.g., the preference of EC10 over

NOEC). This has resulted in larger bioavailability model application ranges compared with those of the original chronic Cu bioavailability model set.

The three updated chronic Cu bioavailability models cover the three trophic levels (i.e., invertebrates, vertebrates, and algae) that are required for the use of bioavailability models in environmental threshold derivations in Europe (European Chemicals Agency, 2008; EU Water Directors, 2018, 2021). Evidence of cross-species applicability is a prerequisite for the use of bioavailability models for metals in risk assessment frameworks under REACH or the PPPR (European Chemicals Agency [2008] and EFSA [2021], respectively) or EQS settings under the WFD (EU Water Directors, 2018, 2021). In the present study, we demonstrated the cross-species application of the updated models to nonmodel cladocerans, rotifers, algae, and plants (Table 2). Recently, Nys et al. (2020) proved that the updated chronic Cu fish bioavailability model developed based on juvenile rainbow trout (*O. mykiss*) can be extrapolated to other life stages of the same species as well of other fish species (i.e., *P. promelas*). For a few taxonomic groups there remains some uncertainty related to the cross-species applicability of the updated chronic Cu invertebrate bioavailability model. This is the case for amphipods because the model prediction performance differed between the two included subchronic *H. azteca* data sets. Overall, the evidence suggests that up to a pH of 7.7 the updated Cu invertebrate bioavailability model can be used to predict Cu toxicity to *H. azteca* with reasonable accuracy. For molluscs, there is evidence of the applicability of the bioavailability models based on acute Cu toxicity for sensitive species and endpoints, although evidence from truly chronic data sets is lacking. The only taxonomic group represented in the Cu toxicity database (European Copper Institute, 2022) for which the applicability of the updated invertebrate bioavailability model was not evaluated are the insects. Overall, there are limited studies on chronic metal toxicity to insects (Brix et al., 2011), and to our knowledge there are no studies investigating bioavailability effects on chronic Cu toxicity for this taxonomic group. However, as in the Cu-VRAR, cross-species applicability of the updated bioavailability models toward insects is assumed to be most likely. Whether the sensitivity of insects to copper is important for evaluating environmental threshold levels is unclear based on present reports. Brix et al. (2011) reported that chronic toxicity measured in laboratory experiments with insects is generally higher than the European Union 5% hazardous concentration. However, in field studies that consider prolonged exposure some insect taxa appear to be more sensitive than reflected in typical laboratory experiments (Brix et al., 2011). Recent research using insect community stream mesocosms has also suggested that during prolonged exposure, the sensitivity of insect species may vary highly. In addition, the present study identified mayfly species as among the most sensitive species in the species sensitivity distribution (Mebane et al., 2020).

Given the above discussed considerations, but also acknowledging the remaining data gaps, the updated model set can be considered as more robust than the original bioavailability model set for implementation of Cu bioavailability in regulatory assessments. To underpin the safe use of Cu, the updated bioavailability model set (although including some minor differences in S_{pH} slopes) was therefore used by the REACH Copper Consortium in 2022 to update the Cu PNEC under the European Union REACH regulation (European Copper Institute, 2022).

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5796.

Acknowledgments—The present study was funded by the International Copper Association. The authors wish to thank T. Van Regenmortel for her contribution to earlier versions of the developed models.

Conflict of Interest—The authors are subcontractors or work for the International Copper Association.

Author Contributions Statement—**Charlotte Nys**: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Visualization; Writing—original draft; Writing—review & editing. **Stephen Lofts**: Software; Writing—review & editing. **Patrick Van Sprang**: Supervision; Writing—review & editing. **Stijn Baken**: Supervision; Writing—review & editing. **Katrien Delbeke**: Writing—review & editing. **Karel De Schamphelaere**: Conceptualization; Methodology; Writing—review & editing.

Data Availability Statement—The raw data and associated speciation databases used for this assessment are provided as Supplementary Information to the manuscript.

REFERENCES

Ahmed, I. A. M., Hamilton-Taylor, J., Bieroza, M., Zhang, H., & Davison, W. (2014). Improving and testing geochemical speciation prediction of metal ions in natural waters. *Water Research*, *67*, 276–291.

Ahmed, I. A. M., Hamilton-Taylor, J., Lofts, S., Meeussen, J. C. L., Lin, C., Zhang, H., & Davison, W. (2013). Testing copper-speciation predictions in freshwaters over a wide range of metal-organic matter ratios. *Environmental Science & Technology*, *47*, 1487–1495.

Antunes, P. M., Scornalenchi, M. L., & Roshon, H. D. (2012). Copper toxicity to *Lemna minor* modelled using humic acid as a surrogate for the plant root. *Chemosphere*, *88*, 389–394.

Borgmann, U., Nowierski, M., & Dixon, D. G. (2005). Effects of major ions on the toxicity of copper to *Hyalella azteca* and implications for the biotic ligand model. *Aquatic Toxicology*, *73*, 268–287.

Brix, K. V., DeForest, D. K., & Adams, W. J. (2011). The sensitivity of aquatic insects to divalent metals: A comparative analysis of laboratory and field data. *Science of the Total Environment*, *409*, 4187–4197.

Brix, K. V., DeForest, D. K., Tear, L., Grosell, M., & Adams, W. J. (2021). Use of multiple linear regression models for setting water quality criteria for copper: A complementary approach to the biotic ligand model. *Environmental Science and Technology*, *51*, 5182–5192.

Crémazy, A., Wood, C. M., Ng, T. Y., Smith, D. S., & Chowdhury, M. J. (2017). Experimentally derived acute and chronic copper Biotic Ligand Models for rainbow trout. *Aquatic Toxicology*, *192*, 224–240.

Deaver, E., & Rodgers, J. (1996). Measuring bioavailable copper using anodic stripping voltammetry. *Environmental Toxicology and Chemistry*, *15*, 1925–1930.

De Schamphelaere, K. A. C., & Janssen, C. R. (2002). A biotic ligand modelpredicting acute copper toxicity for *Daphnia magna*: The effects of calcium, magnesium, sodium, potassium and pH. *Environmental Science and Technology*, *36*, 48–54.

De Schamphelaere, K. A. C., & Janssen, C. R. (2004). Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*. *Environmental Toxicology and Chemistry*, *23*, 1365–1375.

De Schamphelaere, K. A. C., & Janssen, C. R. (2006). Bioavailability models for predicting copper toxicity to freshwater green microalgae as a function of water chemistry. *Environmental Science and Technology*, *40*, 4515–4522.

De Schamphelaere, K. A. C., & Janssen, C. R. (2008). Chapter 3—Appendix U: Modelling copper bioavailability and toxicity in freshwater: Uncertainty reduction for risk assessment (Chronic fish-BLM). In: *European Union risk assessment report: Voluntary risk assessment of copper, copper ii sulphate pentahydrate, copper(i)oxide, copper(ii) oxide, dicopper chloride trihydroxide* (pp. 1–35). European Copper Institute.

De Schamphelaere, K. A. C., Heijerick, D. G., & Janssen, C. R. (2006). Cross-phylum comparison of a chronic biotic ligand model to predict chronic toxicity of copper to a freshwater rotifer; *Brachionus calyciflorus* (Pallas). *Ecotoxicology and Environmental Safety*, *63*(2), 189–195.

De Schamphelaere, K. A. C., Vasconcelos, F. M., Heijerick, D. G., Tack, F. M. G., Delbeke, K., Allen, H. E., & Janssen, C. R. (2003). Development and field validation of a predictive copper toxicity model for the green alga *Pseudokirchneriella subcapitata*. *Environmental Toxicology and Chemistry*, *22*, 2454–2465.

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*, 2383–2396.

Erickson, R. J., Benoit, D. A., Mattson, V. R., Nelson, Jr., H. P., & Leonard, E. N. (1996). The effects of water chemistry on the toxicity of copper to fathead minnows. *Environmental Toxicology and Chemistry*, *15*, 181–193.

European Chemicals Agency. (2008). *Guidance on information requirements and chemical safety assessment Appendix R.7.13-2: Environmental risk assessment for metals and metal compounds*.

European Copper Institute. (2008). *Voluntary risk assessment reports on copper and copper compounds: European Copper Institute consolidated reports, TCNES opinions and SCHER opinions*. Retrieved July 9, 2023, from: https://echa.europa.eu/nl/copper-voluntary-risk-assessment-reports

European Copper Institute. (2022). *REACH registrations by the copper consortium*. Retrieved July 9, 2023, from: https://reach-copper-consortium.eu/substances/

European Food Safety Authority Plant Protection Regulation Panel. (2021). Statement of the PPR Panel on a framework for conducting the environmental exposure and risk assessment for transition metals when used as active substances in plant protection products (PPP). *EFSA Journal*, *19*(3), 6498.

EU Water Directors. (2018). Guidance document No. 27: Technical Guidance for deriving environmental quality standards. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Updated version 2018.

EU Water Directors. (2021). Guidance document No. 38. Technical Guidance for implementing environmental quality standards (EQS) for metals. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Retrieved July 9, 2023, from: https://circabc.europa.eu/ui/group/9ab5926d-bed4-4322-9aa7-9964bbe8312d/library/a705289f-7001-4c7d-ac7c-1cf8140e2117/details

Garman, E. R., Meyer, J. S., Bergeron, C. M., Blewett, T. A., Clements, W. H., Elias, M. C., Farley, K. J., Gissi, F., & Ryan, A. C. (2020). Validation of bioavailability-based toxicity models for metals. *Environmental Toxicology and Chemistry*, *39*, 101–117.

Grosell, M., McDonald, M. D., Walsh, P. J., & Wood, C. M. (2004). Effects of prolonged copper exposure in the marine gulf toadfish (*Opsanus beta*). II. Drinking rate, copper accumulation and Na+/K+-ATPase activity in osmoregulatory tissues. *Aquatic Toxicology*, *68*, 263–275.

Heijerick, D., Bossuyt, B., & Janssen, C. (2002). Euro-Ecole: Assessment of the Bioavailability and Potential Ecological Effects of Copper in European Surface Waters. Subproject 4: Evaluation and improvement of the ecological relevance of laboratory generated toxicity data. Report submitted to the European Copper Institute.

Heijerick, D. G., Bossuyt, B. T. A., De Schamphelaere, K. A. C., Indeherberg, M., Mingazinni, M., & Janssen, C. R. (2005). Effects of varying physicochemistry of European surface waters on the copper toxicity to the green algae *Pseudokirchneriella subcapitata*. *Ecotoxicology*, *14*, 661–670.

International Organization for Standardization. (2013). *Water quality—Determination of toxicity of fresh water sediments using* Hyalella azteca. ISO 16303.

Ivey, C. D., Ingersoll, C. G., Brumbaugh, W. G., Hammer, E. J., Mount, D. R., Hockett, J. R., Norberg-King, T. J., Soucek, D., & Taylor, L. (2016). Using an interlaboratory study to revise methods for conducting 10-d to 42-d water or sediment toxicity tests with *Hyalella azteca*. *Environmental Toxicology and Chemistry*, *35*, 2439–2447.

Kandegedara, A., & Rorabacher, D. B. (1999). Noncomplexing tertiary amines as "better" buffers covering the range of pH 3–11. Temperature dependence of their acid dissociation constants. *Analytical Chemistry*, *71*(15), 3140–3144.

Lavoie, M., Le Faucheur, S., Boullemant, A., Fortin, C., & Campbell, P. G. C. (2012). The influence of pH on algal cell membrane permeability and its implications for the uptake of lipophilic metal complexes. *Journal of Phycology*, *48*, 293–302.

Lofts, S., & Tipping, E. (2008). The chemical speciation of Fe(III) in freshwaters. *Aquatic Geochemistry*, *14*, 337–358.

Lofts, S., & Tipping, E. (2011). Assessing WHAM/Model VII against field measurements of free metal ion concentrations: Model performance and the role of uncertainty in parameters and inputs. *Environmental Chemistry*, *8*, 501–516.

Markich, S. J., Brown, P. L., Jeffree, R. A., & Lim, R. P. (2003). The effects of pH and dissolved organic carbon on the toxicity of cadmium and copper to a freshwater bivalve: Further support for the extended free ion activity model. *Archives of Environmental Contamination and Toxicology*, *45*(4), 479–491.

Mebane, C. A. (2023). Bioavailability and toxicity models of copper to freshwater life: The state of regulatory science. *Environmental Toxicology and Chemistry*, *42*, 2529–2563.

Mebane, C. A., Schmidt, T. S., Miller, J. L., & Balistrieri, L. S. (2020). Bioaccumulation and toxicity of cadmium, copper, nickel and zinc and their mixtures to aquatic insect communities. *Environmental Toxicology and Chemistry*, *39*, 812–833.

Messiaen, M., Janssen, C. R., De Meester, L., & De Schamphelaere, K. A. C. (2013). The initial tolerance to sub-lethal Cd exposure is the same among ten naïve pond populations of *Daphnia magna*, but their micro-evolutionary potential to develop resistance is very different. *Aquatic Toxicology*, *144–145*, 322–331.

Nys, C., Janssen, C. R., Van Sprang, P., & De Schamphelaere, K. A. C. (2016). The effect of pH on chronic aquatic Ni toxicity is dependent on the pH itself: Extending the chronic Ni bioavailability models. *Environmental Toxicology and Chemistry*, *35*, 1097–1106.

Nys, C., Van Sprang, P., & De Schamphelaere, K. A. C. (2020). A generalized bioavailability model (gBAM) for predicting chronic copper toxicity to freshwater fish. *Environmental Toxicology and Chemistry*, *39*, 2424–2436.

Peters, A., Merrington, G., De Schamphelaere, K., & Delbeke, K. (2011). Regulatory consideration of bioavailability for metals: Simplification of input parameters for the chronic copper biotic ligand model. *Integrated Environmental Assessment and Management*, *7*, 437–444.

Peters, A., Nys, C., Merrington, G., Verdonck, F., Baken, S., Copper, C. A., Van Assche, F., Schlekat, C., & Garman, E. (2020). Demonstrating the reliability of bio-met for determining compliance with environmental quality standards for metals in Europe. *Environmental Toxicology and Chemistry*, *39*, 2361–2377.

Peters, A., Nys, C., Leverett, D., Wilson, I., Van Sprang, P., Merrington, G., Middleton, E., Garman, E., & Schlekat, C. (2023). Updating the chronic freshwater ecotoxicity database and biotic ligand model for nickel for regulatory applications in Europe. *Environmental Toxicology and Chemistry*, *42*, 566–580.

Playle, R. C., & Wood, C. M. (1989). Water chemistry changes in the gill micro-environment of rainbow trout: Experimental observations and theory. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology*, *159*, 527–537.

Ritchie, J. D., & Perdue, E. M. (2003). Proton-binding study of standard and reference fulvic acids, humic acids, and natural organic matter. *Geochimica et Cosmochimica Acta*, *67*(1), 85–96.

Rodriguez, P. H., & Arbildua, J. J. (2012). *Copper acute and chronic toxicity to D. magna: Sensitivity at three different hardness at pH 6.3 (MES buffered) in the presence of 2 mg/L DOC*. Report submitted to the International Copper Association (ICA).

Schwartz, M. L., & Vigneault, B. (2006). Development and validation of a chronic copper biotic ligand model for *Ceriodaphnia dubia*. *Aquatic Toxicology*, *84*(2), 247–254.

Tao, S., Long, A., Fuliu, X., & Dawson, R. W. (2002). Copper speciation in the gill microenvironment of carp (*Cyprinus carpio*) at various levels of pH. *Ecotoxicology and Environmental Safety*, *52*, 221–226.

Tipping, E. (2002). *Cation binding by humic substances. Cambridge environmental chemistry series* (p. 448). Cambridge University Press.

Tipping, E., Lofts, S., & Sonke, J. E. (2011). Humic Ion-Binding Model VII: A revised parameterisation of cation binding by humic substances. *Environmental Chemistry*, *8*(3), 302–325.

Van Regenmortel, T. (2017). *Ecotoxicity and risk assessment of metal mixtures in the freshwater environment*. Doctoral thesis submitted, Applied Biological Sciences, Ghent University, Gent, Belgium.

Van Regenmortel, T., Janssen, C. R., & De Schamphelaere, K. A. C. (2015). Comparison of the capacity of two biotic ligand models to predict chronic copper toxicity to two *Daphnia magna* clones and formulation of a generalized bioavailability model. *Environmental Toxicology and Chemistry*, *34*, 1597–1608.

Van Sprang, P., Nys, C., Blust, R., Chowdury, J., Gustafsson, J. P., Janssen, C. R., & De Schamphelaere, K. A. C. (2016). The derivation of effects threshold concentrations of Pb for European freshwater ecosystems. *Environmental Toxicology and Chemistry*, *35*, 1310–1320.

Villavicencio, G., Urrestarazu, P., Arbildua, J., & Rodriguez, P. H. (2011). Application of an acute biotic ligand model to predict chronic copper toxicity to *Daphnia magna* in natural water of Chile and reconstituted synthetic waters. *Environmental Toxicology and Chemistry*, *30*, 19–25.

Wang, N., Ingersoll, C. G., Greer, I. E., Hardesty, D. K., Ivey, C. D., Kunz, J. L., Brumbaugh, W. G., Dwyer, F. J., Roberts, A. D., Augspurger, T., & Kane, C. M. (2007). Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry*, *26*(10), 2048–2056.

Wang, N., Mebane, C. A., Kunz, J. L., Ingersoll, C. G., May, T. W., Arnold, W. R., Santore, R. C., Augspurger, T., Dwyer, F. J., & Barnhart, M. C. (2009). Evaluation of acute copper toxicity to juvenile freshwater mussels (fatmucket, *Lampsilis siliquoidea*) in natural and reconstituted waters. *Environmental Toxicology and Chemistry*, *28*(11), 2367–2377.

Wang, N., Mebane, C. A., Kunz, J. L., Ingersoll, C. G., Brumbaugh, W. G., Santore, R. C., Gorsuch, J. W., & Arnold, W. R. (2011). Influence of dissolved organic carbon on toxicity of copper to a uniod mussel (*Villosa iris*) and a cladoceran (*Ceriodaphnia dubia*) in acute and chronic water exposures. *Environmental Toxicology and Chemistry*, *30*, 2115–2125.

Wilde, K. L., Stauber, J. L., Markich, S. J., Franklin, N. M., & Brown, P. L. (2006). The effect of pH on the uptake and toxicity of copper and zinc in a tropical freshwater alga (*Chlorella* sp.). *Archives of Environmental Contamination and Toxicology*, *51*, 174.

