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Metals Cooperative Research and Development Agreement (CRADA) Phase I Report:

Development of an Overarching Bioavailability Modeling Approach to Support US EPA's Aquatic Life Water Quality Criteria for Metals

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**Developed by the US Environmental Protection Agency
in collaboration with the Metals CRADA Partners**

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- B. Explanation of How Toxicity Modifying Factors (TMFs) Affect Individual Metals developed by CRADA Partners
- C. Table 1: Bioavailability Model Comparisons, Table 2: Supporting Information, and References developed by CRADA Partners. See supplemental materials section at <https://www.epa.gov/wqc/metals-crada-phase-1-report> .
- D. Aluminum and Copper model comparisons: Brix et al. 2020b peer reviewed internal report. The copper portion of this analyses has been published and is available at <https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5012>. The publication for the aluminum MLR portion of this analyses has been published and is available at <https://setac.onlinelibrary.wiley.com/doi/epdf/10.1002/etc.4796>. Any subsequent publications will be listed when available in the supplemental materials section at <https://www.epa.gov/wqc/metals-crada-phase-1-report> .

- E. Lead model comparison: DeForest et al. 2020b peer reviewed internal report. The publication for the lead analyses is in preparation and will be listed when available in the supplemental materials section at <https://www.epa.gov/wqc/metals-crada-phase-1-report> .
 - F. Nickel model comparison: Santore et al. 2020 peer reviewed internal summary report. Santore et al. 2021 and Croteau et al. 2021 have been published and are available at <https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5109> and <https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.5063>, respectively.
 - G. Biotic Ligand Models and Multiple Linear Regression models provided by the CRADA Partners for comparison with artificial and natural waters (internal appendix containing proprietary modeling information which includes the software needed to run the models for aluminum, copper, lead, and nickel and databases with sample water quality parameters and answer keys).
- Addendum: Summary of How Toxicity Modifying Factors (TMFs) Affect Metals Listed in Table 1. Information provided through the peer review by Christopher A. Mebane (USGS).

I. Overview of the Metals CRADA Project

In December 2017, the U.S. Environmental Protection Agency (EPA) signed a Cooperative Research and Development Agreement (CRADA) with eight metals associations (Aluminum Association, Aluminum REACH Consortium, Cobalt Institute, International Copper Association, Copper Development Association, International Lead Association, International Zinc Association, NiPERA Inc.) in order to leverage the knowledge and resources of scientists inside and outside of the agency to better protect aquatic life. EPA's Office of Science and Technology within the Office of Water (OW) is the Agency's technical lead on this CRADA which supports *EPA's FY 2018-2022 Strategic Plan* Goal: Provide for Clean and Safe Water: Protect and Restore Water Quality. EPA is using a two-phased approach to address the CRADA. In the first phase, EPA has worked with external technical experts from the metals associations to develop a proposed modeling approach to predict the bioavailability and toxicity of metals under the range of water chemistry conditions found in aquatic environments common in freshwaters of the United States. Subsequently, in the second phase, EPA will work with the metals associations to develop bioavailability models for individual metals using the overarching modeling approach. Using the resulting peer-reviewed models, EPA plans to develop updated, externally-peer reviewed Aquatic Life Ambient Water Quality Criteria for metals to better support states, territories and tribes with criteria that reflect the latest science and are easier to implement than more complex, previous approaches using metals bioavailability modeling for criteria development.

a. Brief overview of metals bioavailability

As summarized in Adams et al. (2020), metal toxicity to aquatic organisms is variable depending on the physicochemical characteristics of the water in which they reside. Adverse effects occur when the metal binds to or accumulates on biotic ligands (surface binding sites leading to internalization and effect, for example, on the gill surface) and reaches a critical toxic threshold. Common water chemistry parameters that are known to affect the toxicity of one or more metals include pH, alkalinity, hardness, temperature, sodium, chloride, suspended solids, and colloidal or dissolved organic carbon (DOC). The bioavailability of metals to aquatic organisms is influenced by these parameters as they control the rate and extent to which the metal reaches the site of action by affecting the solubility, sorption, or partitioning of the metal. The variability in the toxicity of metals as a result of different water chemistries was recognized as early as the 1930s. Since then, research has led to the development of models to describe and predict the toxicity of metals and the response of aquatic organisms at differing water chemistries. Current bioavailability-based models often used to predict metal toxicity include: 1) empirically-based linear regression equations based on single parameters, like hardness, 2) the mechanistically-based Biotic Ligand Model (BLM), and 3) empirically-based multiple linear regression (MLR) models.

b. Overview of EPA’s metals criteria and historic use of bioavailability-based approaches

EPA develops Aquatic Life Ambient Water Quality Criteria (AWQC) for metals pursuant to Clean Water Act Section 304(a)(1). AWQC are intended to protect aquatic organisms from the toxic effects of metals in the aquatic environment and thereby the aquatic life designated use. For most metals, the AWQC is not a single number to be applied uniformly across all surface waters. Early AWQC for metals published in the 1980’s were developed to take into account the effects of ambient water hardness on toxicity. Hardness-based criteria are based on simple linear regression models where the numeric magnitude of the AWQC is normalized to be protective at a given site-specific hardness. Currently, EPA has developed recommended AWQC for 9 metals (aluminum, cadmium, chromium (III and IV), copper, iron, lead, nickel, silver, and zinc). Most of these metals criteria were developed in the 1980’s and 1990’s (<https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table>). Recent updated criteria efforts address bioavailability using different modeling approaches. For example, in 2007, EPA revised the AWQC for Copper (US EPA 2007) to incorporate an acute BLM to account for bioavailability as a function of water chemistry. In 2016, EPA updated the acute and chronic hardness slopes for cadmium with data for several new species in the AWQC for Cadmium (US EPA 2016a) and determined that a more complex modeling approach was not necessary for the criteria update. Lastly, in 2018, EPA revised the Final AWQC for Aluminum (US EPA 2018) which uses MLR models to incorporate three parameters (pH, total hardness, and DOC) to normalize acute and chronic toxicity data to water quality conditions. EPA is now working to update the older metals criteria to reflect the latest scientific knowledge on bioavailability using modeling approaches to incorporate water chemistry parameters in addition to hardness, that can modify the bioavailability and toxicity of metals.

c. Goal of project

The goal of the CRADA project is to develop a simplified, overarching modeling framework to predict the bioavailability of metals considering a common model parameter set, modeling approach and platform to update the remaining metals AWQC. This report provides a review of models that are available to predict the toxicity of metals with respect to the factors that modify toxicity as a function of water chemistry. The report focuses on the performance of BLMs and MLR models for existing data sets for aluminum, copper, lead, and nickel. These datasets were developed to meet the criteria established in the 1985 Guidelines (US EPA 1985) for AWQC development and the models were evaluated using the criteria established in the Society of Environmental Toxicology and Chemistry (SETAC) Technical Workshop, *Bioavailability-Based Aquatic Toxicity Models for Metals*, December 2017 (SETAC 2017). The workshop resulted in a series of articles on “Metal Bioavailability Modeling” that evaluated the performance of models and recommend best practices in the development and use of bioavailability-based values for protection of aquatic life (Adams et al. 2020; Brix et al. 2020a; Garman et al. 2020; Mebane et al. 2020; Schlekot et al. 2020; Van Genderen et al. 2020; see **Appendix A** for references).

II. Metal Toxicity Modifying Factors (TMFs) and their relative importance

In the aquatic environment, the toxicity of metals is dependent on many factors including the individual metal and its chemical speciation, and the duration, magnitude, and route of exposure. The effect of a number of metals on aquatic organisms is not well predicted by the total metal concentration (except for aluminum). Metal bioavailability is a function of many modifying factors that affect the speciation, bioavailability, and toxicity of metals. These factors include pH, water hardness (primarily Ca and Mg ions), alkalinity, temperature, sodium, chloride, fluoride, suspended solids, and DOC. However, the toxicity modifying factors (TMFs) that have received the most attention in terms of bioavailability models are pH, hardness, and DOC (Adams et al. 2020).

Meyer et al. (2007) described two ways in which these modifying factors can affect whether metals result in bioavailable concentrations that can cause toxicity by affecting the physiological responses of aquatic organisms. The first is by complexing or sorbing to metal ions (e.g., DOC, carbonates, chloride, and hydroxide) which decreases the concentration of the free metal ion and negatively affects the interaction with binding sites on the organism. The second way is by competing with metal ions for binding sites on organisms (e.g., competition from H^+ , Ca^{2+} , and Mg^{2+}).

Specifically, the effects of the most commonly studied TMFs are described below (see Meyer et al. 2007 for more information and **Appendix B** for more detailed information on how TMFs affect aluminum, copper, lead, nickel and zinc:

a. pH

There are several mechanisms by which changes in H^+ ion concentrations (reflected by changes in pH) can affect metal bioavailability, including speciation, solubility, and competitive interactions between the metal and biotic ligands. The relative effect of H^+ ions depends on the binding strength of the metal to carbonate, bicarbonate and hydroxide ions. Generally, metals dissociate at low pH (less than pH 6 to 7) which increases their solubility and thus bioavailability and toxicity. However, as pH increases above pH 6 to 7, alkalinity often increases as well and many metals become less bioavailable and less toxic because they form complexes with carbonates and hydroxides, and subsequently may precipitate as oxides and hydroxides. Complexation and precipitation reactions, mediated by changes in pH, can therefore affect the concentration of the free metal ions available to bind to the biotic ligand (Meyer et al. 2007).

b. Hardness

In freshwater, hardness is dominated by Ca^{2+} and Mg^{2+} ions which compete with divalent metal ions for binding to the biotic ligand. As a result, increased water hardness generally leads to less metal accumulation by aquatic organisms and lower toxicity. There are differences in the protective effects of Ca^{2+} and Mg^{2+} ions: generally, Ca^{2+} is more protective in fish than Mg^{2+} (Meyer et al. 2007).

c. Dissolved Organic Carbon [DOC]

Dissolved organic matter, typically quantified as DOC, is a heterogeneous mix of organic matter of natural and anthropogenic origin that is impermeable to biological membranes. Generally, an increase in DOC decreases metal bioavailability and toxicity by complexing with free metal ions, thereby reducing metal binding at the biotic ligand. The protective effects of DOC depend on its concentration, composition, and the binding affinity of the metal (Meyer et al. 2007, Wood et al. 2011).

d. Other

Although the TMFs pH, hardness, and DOC have been studied the most, other factors are known also to modify the bioavailability and toxicity of metals. Temperature is potentially an important TMF for some metals, but this is dependent on the species as well as the metal in the exposure scenario. For example, the kinetics underlying aluminum bioavailability has a strong dependency on temperature (Santore et al. 2018). Existing data for other metals such as nickel, copper, and zinc do not show the same magnitude of correlations between temperature and chronic toxicity (Pereira et al. 2017), but more information is needed. Ultimately, this factor has not received enough attention in toxicity testing (Brix et al. 2020a; Mebane et al. 2020) to incorporate this parameter into many models. Another parameter that is potentially important is total suspended solid (TSS). Generally, toxicity decreases as TSS concentration increases because the free metal ion binds to or sorbs to particles. Another parameter that has been investigated is sodium (Na^+). An increase in Na^+ cations generally decreases toxicity by competition at metal binding sites; however, based on the few comparative studies for Cu and Zn toxicity to freshwater invertebrate and algal species, Na^+ appears to provide less protection than Ca^{2+} and Mg^{2+} (Meyer et al. 2007).

As mentioned above, metals respond differently to the effects of various TMFs which, in part, is dependent on the type and strength of bonds (ionic or covalent) formed with the binding sites (Meyer et al. 2007). **Table 1** illustrates the relative importance of the most studied TMFs for several metals within a given metal (not across metals). This table is a general guideline as these trends may be variable depending on the species, life stage, test duration, and other factors that are considered within bioavailability models.

Table 1. Toxicity modifying factors that have been demonstrated to be important in various BLM and MLR published models and their relative importance within each metal.

Metal	Type	Most Important Parameters ¹			
		Hardness	pH	DOC	Other
Aluminum	Freshwater	+	+++	++	temperature
Cadmium	Freshwater	+++	+	+	
Cobalt	Freshwater	++	+	+	
Copper	Freshwater	+	++	+++	sodium
Copper	Marine		+	+	salinity
Lead	Freshwater	+	+	+++	
Nickel	Freshwater	+		+	
Silver	Freshwater			+	chromium reducible sulfur, sodium, chloride
Zinc	Freshwater	+++	++	+	

¹Since it is difficult to separate the effects of alkalinity and pH, alkalinity is not listed as a separate factor but is considered as a contribution to the overall effects of pH.

See **Appendix B** for a detailed summary of the how TMFs affect some of the metals (aluminum, copper, lead, nickel and zinc) listed in Table 1. See also **Appendix H** for a high-level summary of how TMFs affect the metals listed in Table 1.

It is important that high quality TMF data be collected for the use in bioavailability model development or as input parameters into the model. Data should be collected using good sampling and measurement practices, particularly in regard to pH and DOC collection (e.g., Balistrieri et al. 2012, Nimick et al. 2011, and Yoro et al. 1999).

III. Discussion of bioavailability modeling approaches examined

Bioavailability-based models have been developed to take the influence of water chemistry into account when evaluating aqueous metal toxicity to aquatic organisms. Diet is another route of metal exposure that is generally not considered within bioavailability models because of a lack of available data and mechanistic complexity. Currently, for most metals, data indicate that respiratory organs are more sensitive to cationic metals via water exposure than exposure through the gut. Furthermore, these models have been validated with long-term mesocosm studies in which the dietary route of exposure is an operational pathway (Roussel et al. 2007; Schlekat et al. 2010; Versteeg et al. 1999). Additionally, in a dietary zinc toxicity study, De Schampelaere et al. (2004) concluded that “the zinc BLM predicts chronic reproductive zinc bioavailability and toxicity in synthetic and field surface waters with reasonable accuracy even without explicitly directly considering the dietary toxicity pathway.” For many metals, toxicity

stemming from the waterborne pathway has been shown to occur at similar or lower concentrations than the dietary route (e.g., Evens et al. 2009 for nickel, De Schamphelaere et al. 2007 for copper, Nys et al. 2013 and Alsop et al. 2016 for lead), indicating that AWQC which are protective of aqueous metal exposure are also protective of dietary exposures. Mebane et al. (2020) also suggested there is currently “insufficient evidence to conclude that bioavailability models would be under-protective if based on waterborne-only exposures” and recommended that researchers conduct concurrent exposures to strengthen the literature surrounding dietary exposure and support the development of a biodynamic modeling framework that is able to incorporate the dietary exposure route (Mebane et al. 2020). Lastly, when it is well-established that the diet is an important exposure route, EPA has considered this information in their criteria development. For example, the freshwater selenium water quality criteria (US EPA 2016b) are based on fish tissue concentrations since diet is the primary route of exposure.

The approaches used to develop bioavailability-based models fall within a continuum between empirical (e.g., hardness equations) and mainly mechanistic (e.g., biokinetic BLM) (see Textbox 3 in Adams et al. 2020 and Figure 1 in Brix et al. 2020a). In the middle of the continuum are the empirically-based MLR and mechanistically-based BLM. Adams et al. (2020) and Mebane et al. (2020) provide overviews of the history of the science resulting in the development of the BLM and later MLR models, as well as other bioavailability models not under consideration as an overarching approach at this time as they are either not as scientifically robust and/or practical as the BLM and MLR models (e.g., hardness-based equations, WER, generalized bioavailability models [gBAMs] and biodynamic models). In addition, after reviewing bioavailability-based toxicity models in terms of use, refinement, and application to protection values, Mebane et al. (2020) lays out a series of recommendations for developing mechanistically-based models. Similarly, Brix et al. (2020a) describe best practices for the development and evaluation of empirical models.

In this section, we describe the BLM and MLR approaches and discuss the advantages and disadvantages of each, which can depend on the complexity of the environmental chemistry, data availability and intended use or policy decisions for a given metal. **Table 2** highlights the different models in this category that are currently available or in development for the six metals represented by the CRADA (Al, Co, Cu, Pb, Ni and Zn). Bioavailability models encompassed in this table span across fresh- and marine waters and, in total, include 17 BLMs and 13 MLR models developed across different global jurisdictions. Simplified bioavailability look-up tools (e.g., Bio-Met, M-BAT), which have been designed for regulatory ease-of-use, are also included in this comparison framework. More information is provided in **Appendix C** where the comparative metrics have been divided into two tables. The “Primary” comparison table, similar to **Table 2**, summarizes major details of each model including the user-interface, primary toxicity modifying factors and chemistry inputs required for each model, the output value generated and the source/references from which the model can be obtained. The “Supplemental” comparison table describes specific details surrounding the development of the models such as applicable chemistry ranges, validation datasets, and the use in regulatory frameworks. The “References” table contains full references for all information included in the primary and supplemental tables.

Table 2: Comparisons of bioavailability models currently available or in development for the six metals represented by the CRADA. More information is provided in **Appendix C** which also summarizes other major details of each model including the user-interface, the output value generated and the source/references from which the model can be obtained. An additional table describes specific details surrounding the development of the models such as applicable chemistry ranges, validation datasets, and the use in regulatory frameworks. Reference list provided in **Appendix C**.

Metal	Model	Type	Primary toxicity modifying factors	Taxa model is applicable to	Chemistry Inputs needed
Aluminum	BLM v3.18.2.42	Full BLM	DOC, Hardness, pH, Temperature	A, I, F	Temperature, pH, DOC, Al, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity
	MLR	MLR	DOC, Hardness, pH	A, I, F	pH, DOC, Hardness
Cobalt	BLM v3.15.2.41	Full BLM	DOC, Hardness, pH	A, I, F	Temperature, pH, Co, DOC, Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
	MLR	MLR	DOC, Hardness, pH	A, I, F	pH, DOC, Hardness (Ca, Mg)
	Bio-met v5.1	Simplified BLM Lookup Tool	DOC, Ca, pH	A, I, F	pH, DOC, Ca, Co
Copper	USEPA BLM	Full BLM	Alkalinity, DOC, Hardness, pH	I, F	Temperature, pH, Cu, DOC, Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
	ECCC BLM v1.10	Full BLM	Alkalinity, DOC, Hardness, pH	A, P, I, F	Required: Temperature, pH, Cu, DOC, Hardness; Optional: Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
	BC BLM v1.11	Full BLM	Alkalinity, DOC, Hardness, pH	A, P, I, F, Am	Required: Temperature, pH, Cu, DOC, Hardness; Optional: Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
	Windward BLM v3.41.2.45	Full BLM	Alkalinity, DOC, Hardness, pH	I, F	Required: Temperature, pH, Cu, DOC, Hardness; Optional: Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S

Metal	Model	Type	Primary toxicity modifying factors	Taxa model is applicable to	Chemistry Inputs needed
Copper	BLM/gBAM	Mixed regression + speciation model	Alkalinity, DOC, Hardness, pH	I, F	Temperature, pH, Cu, DOC, Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
	Bio-met v5.0	Simplified BLM Lookup Tool	DOC, Ca, pH	A, I, F	pH, DOC, Ca, Cu
	M-BAT v30.0	Simplified BLM Lookup Tool	DOC, Ca, pH	A, I, F	pH, DOC, Ca, Cu
	PNEC-Pro v6.0	[M]LR	DOC	A, I, F	Required: DOC; Optional: pH, Mg, Ca, Na, Cu
	WHAM- <i>F</i> _{TOX}	Toxicity model linked to speciation	Not specified	P	Temperature, pH, Cu, DOM (fulvic and humic acids), Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, metals
	MLR	MLR	DOC, Hardness, pH	I, F	pH, Hardness, DOC
	Windward Marine BLM v3.41.2.45	Full BLM	DOC, pH, salinity	I, F	Required: Temperature, pH, Cu, DOC, Salinity; Optional: Ca, Mg, Na, K, SO ₄ , Cl, PO ₄ , DIC
	Marine MLR	[M]LR	DOC	I (<i>Mytilus</i> sp.)	DOC
Lead	Unified/North America BLM	Full BLM	DOC, Hardness, pH	I, F	Temperature, pH, Pb, DOC, Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
	EU Risk Assessment BLM/gBAM	Full BLM	DOC, Hardness, pH	A, I, F	Temperature, pH, Pb, DOC, Ca, Mg, Na, K, SO ₄ , Cl, CO ₃
	EU Risk Assessment Lead EQS Screening Tool v1.0	DOC Equation	DOC	A, I, F	DOC, Pb

Metal	Model	Type	Primary toxicity modifying factors	Taxa model is applicable to	Chemistry Inputs needed
Lead	Bio-met v5.0	Simplified BLM Lookup Tool	pH, DOC, Ca	A, I, F	pH, DOC, Ca, Pb
	PNEC Pro v6.0	[M]LR	DOC	A, I, F	Required: DOC; Optional: pH, Mg, Ca, Na, Pb
	Canadian WQG MLR	MLR	DOC, Hardness, pH	A, I, F	pH, DOC, Hardness
	MLR	MLR	DOC, Hardness, pH	A, I, F	pH, DOC, Hardness
Nickel	EU Risk Assessment BLM	Full BLM	DOC, Hardness, pH	A, I, F	Temperature, pH, Ni, DOC, Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
	Bio-met v5.0	Simplified BLM Lookup Tool	pH, DOC, Ca	A, I, F	pH, DOC, Ca, Ni
	M-BAT 20150206	Simplified BLM Lookup Tool	pH, DOC, Ca	A, I, F	pH, DOC, Ca, Ni
	Best Overall Pooled	Full BLM	DOC, Hardness, pH	A, I, F	Temperature, pH, Ni, DOC, Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
	North American <i>C. dubia</i> BLM	Full BLM	DOC, Hardness, pH, Alkalinity	I	Temperature, pH, Ni, DOC, Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
	PNEC Pro v6.0	[M]LR	DOC, Hardness, pH	A, I, F	Required: DOC; Optional: pH, Mg, Ca, Na, Ni
	MLR	MLR	DOC, Hardness	A, I, F	Hardness (Ca/Mg), DOC, pH, Ni
	Marine BLM	Full BLM	DOC	I	Temperature, pH, Ni, DOC, Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
Zinc	Unified/North America BLM	Full BLM	DOC, Hardness, pH	I, F	Temperature, pH, Zn, DOC, Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S
	EU Risk Assessment BLM/gBAM	Full BLM	DOC, Hardness, pH	A, I, F	Temperature, pH, Zn, DOC, Humic acid %, Ca, Mg, Na, K, SO ₄ , Cl, Alkalinity, S

Metal	Model	Type	Primary toxicity modifying factors	Taxa model is applicable to	Chemistry Inputs needed
Zinc	M-BAT v30.0 - 20150206	Simplified BLM Lookup Tool	pH, DOC, Ca	A, I, F	pH, DOC, Ca, Zn
	Bio-met v5.0	Simplified BLM Lookup Tool	pH, DOC, Ca	A, I, F	pH, DOC, Ca, Zn
	PNEC Pro v6.0	[M]LR	DOC	A, I, F	Required: DOC; Optional: pH, Mg, Ca, Na, Zn
	Canadian WQG MLR	MLR	DOC, Hardness, pH	A, I, F	pH, DOC, Hardness
	MLR	MLR	DOC, Hardness, pH	A, I, F	pH, DOC, Hardness
	Marine BLM	Full BLM	DOC, pH, salinity	I, F	Required: Temperature, pH, Zn, DOC, Salinity; Optional: Ca, Mg, Na, K, , Cl, PO ₄ , DIC

Model name, Version/Identification, and Type abbreviations: BC - British Columbia; BLM - Biotic ligand model; ECCC - Environment and Climate Change Canada; EU - European Union; gBAM - Generalized bioavailability model; M-BAT - Metal bioavailability assessment tool; MLR - Multiple linear regression; [M]LR - Multiple linear regression and/or simple linear regression; PNEC - Predicted no effect concentration; USEPA - United States Environmental Protection Agency; WHAM - Windermere humic aqueous model; WQG - Water quality guideline.

Taxa model applicable to abbreviations: A - algae; I – invertebrates; F – fish; P – plants; Am – amphibians

Chemistry inputs needed abbreviations: DIC - Dissolved inorganic carbon; DOC - Dissolved organic carbon

a. Biotic Ligand Models

Biotic Ligand Models are mechanistically-based and the most complex of the models considered. As summarized in Adams et al. (2020), the BLM uses sub-models to account for 1) chemical speciation, 2) the competition of metal and non-metal ions and complexes for binding to the biotic ligand (which is assumed to be the gill or respiratory mechanism) and 3) the metal accumulation and toxicity. BLMs require several inputs of water parameters for chemical speciation calculations including: temperature, pH, DOC, major ions (Ca, Mg, Na, K, Cl, SO₄), and alkalinity. Once the water parameters are entered into the model, the BLM predicts the concentration of the different metal species (complexes and free metal ion) associated with a critical accumulation (i.e., an accumulation level at the biotic ligand that corresponds to a certain effect level). BLMs assume that equilibrium is reached immediately and there are no changes in reaction rates over time.

Acute and/or chronic BLMs have been developed for several metals, including all six of the metals represented by the CRADA (see **Table 2, Appendix C**, and Table 2 in Mebane et al. 2020). However, currently only four regulatory jurisdictions have adopted the BLM approach to develop aquatic life protective values (EPA's Cu AWQC [US EPA 2007], British Columbia's Cu Water Quality Guideline for Protection of Freshwater Aquatic Life [B.C. Ministry of Environment and Climate Change Strategy 2019], Environment and Climate Change Canada's draft Cu Federal Environmental Quality Guidelines [ECCC 2019], and European Commission's Ni Environmental Quality Standard [EQS; European Commission 2010]). BLMs are also under consideration by others (Canada and Australia/New Zealand) (Adams et al. 2020). One of the primary advantages of the BLM approach is that it is based on the premise that bioavailability is linked to chemical speciation, which supports its application to a wide range of conditions and media. However, a barrier to adoption and implementation is the complexity of the approach which can be technically demanding, transparency of the algorithms, and the large number of water chemistry parameters required, some of which are costly and not routinely collected. For example, in 2007, EPA finalized its recommended Cu AWQC, however only five states (Delaware, Idaho, Iowa, Kansas, and Oregon) and the Commonwealth of the Northern Mariana Islands have adopted the Cu BLM statewide and nine states (California, Colorado, Georgia, Maryland, Massachusetts, New Hampshire, North Carolina, South Carolina, and Texas) have the ability to develop site-specific Water Quality Standards to be submitted to EPA for review and approval.

b. Simplified Biotic Ligand Models

To address the issues of complexity, transparency, and water chemistry requirements which have hindered the adoption and implementation of the BLM, several simplified or abbreviated tools have been developed based on the full BLM. Most of these "user-friendly" tools have been developed under the European Union's Water Framework Directive (Adams et al. 2020). Compared to the full BLM, these tools require fewer water chemistry input parameters (but typically still require DOC, pH, and hardness [or Ca as an estimator of hardness]) by restricting

input parameters or relying on default values and require less training. Examples of simplified BLMs include Bio-met (an Excel-based “look-up” table with EQS values for thousands of combinations of water chemistries that have been calculated using the full BLM), PNEC-pro and MBAT (both “algorithm”-based tools). Adams et al. (2020) provides an overview of these simplified tools and how they perform compared to the BLM. In addition, **Appendix C** provides a summary of the comparison of these tools (e.g., inputs, outputs) to developed BLMs for the six metals represented in the CRADA.

c. Multiple Linear Regressions Models

Multiple linear regression models have more recently been developed (e.g., Brix et al. 2017, DeForest et al. 2018 and 2020a, Brix et al. 2020a) partially in response to the complexity and high water quality data input requirements of the BLM. As summarized in Adams et al. (2020), MLR models are empirically-based, statistically-derived approaches to incorporating TMFs to predict metal toxicity across a range of water chemistries where there are direct measurements of the influence of water chemistry on metal toxicity. This approach is similar to the simple linear regression hardness-based models, but MLRs take into account multiple TMFs like hardness, pH, and DOC (and their interactions, if necessary) and rely on large empirical toxicity data sets covering wide ranges of water chemistry parameters and ecotoxicology endpoints. Unlike the BLM, MLR models often use hardness as a parameter rather than the concentrations of specific ions (e.g., Ca and Mg). One of the main reasons MLR models use hardness is because most end-users monitor hardness rather than Ca and Mg. One line of evidence that validates the use of hardness instead of Ca and/or Mg concentrations in MLR models is the consistency in the result from cross-validation exercises comparing the BLM and MLR predictions (see **Appendices D, E and F**). In addition, models may be fitted to acute or chronic toxicity data and for single species or pooled into a single model for multiple species.

Brix et al. (2020a) recommend a pooled MLR modeling approach, if feasible, because the pooled version may increase the confidence of applying the model to different species as it is based on more data and it often includes a wider range of TMFs than species-specific models. In a pooled MLR modeling approach, species-specific intercepts account for the variances in species sensitivity. However, determining whether to use a species-specific or pooled model depends on the available data for the metal, metal-specific characteristics and interactions¹ with TMFs, and performance over a broad range of water chemistries. For example, EPA decided to use the individual fish and invertebrate models in the final recommended Al AWQC (US EPA 2018) rather than a pooled model because the chronic toxicity of Al differed considerably between species depending on water chemistry conditions.

Acute and/or chronic MLR models have been developed for several metals, including all six of the metals represented by the CRADA (see **Table 2, Appendix C**, and Table 2 in Brix et al. [2020a]). As noted, EPA adopted vertebrate and invertebrate (unpooled) MLRs for the chronic Al AWQC (US EPA 2018). The MLR approach has also been adopted or is under consideration

¹ MLR models can explicitly evaluate the interactive effects of how TMFs influence each other. For example, pH may influence the speciation of a metal, while the influence of hardness on the bioavailability of the metal varies depending on the pH-dependent speciation of the metal.

by others for water quality standards (Canada and Australia/New Zealand) (Adams et al. 2020, ECCC 2020). Some advantages of the MLR approach are the relative simplicity and transparency of the model, decreased number of input parameters (in comparison to the BLM) resulting in easier data collection, and ease of use while maintaining comparable output (see **Section IV**). The primary disadvantage of the MLR is that it does not explicitly address the effects of metals speciation and the binding affinity of the metal for the biotic ligand receptor within the model, but instead these effects are taken into account in the models based on empirical observations. MLRs can be informed by mechanistic analyses by evaluating MLR models against existing BLMs.

IV. Case Studies of Modeling Approach Comparisons

This section provides a comparative evaluation of BLM and MLR models for several metals. While there are many variations of these models available (see **Table 2** and **Appendix C**), the current analyses start with the same underlying toxicity data sets to facilitate model comparisons. **Table 3** reports the model performance scores which form the basis for the evaluation of the model comparisons from methods developed in the 2017 SETAC Metals Bioavailability Modelling workshop (see publications in **Appendix A**; specifically, Garman et al. 2020) and modified by Brix et al. (2020b; see **Appendix D**). Most MLR models include DOC, hardness, and pH as TMFs with the exception of Ni (that only considers DOC and hardness). In addition, these case studies followed EPA guidelines (US EPA 1985) to generate estimated AWQC based on the output of the differing modeling approaches to assess how the criteria respond to changing water quality characteristics.

Table 3: Acute and chronic performance scores for each metal based on the recommended MLR models and BLMs in the cases studies (**Appendices D, E and F**). Performance score is the arithmetic mean of individual scores for adjusted R^2 (for MLR) or R^2 (for BLM), $RF_{x,2.0}$, and residuals (see Garman et al. 2020 and Brix et al. 2020b for details). NA – no model available.

Metal	Acute Score		Chronic Score		Reference
	MLR	BLM	MLR	BLM	
Aluminum	NA	NA	0.91	0.75	Brix et al. 2020b
Copper	0.71	0.71	0.87	0.55	Brix et al. 2020b
Lead	0.79	0.76	0.81	0.62	DeForest et al. 2020b
Nickel	0.90	0.93	0.89	0.88	Croteau et al. 2021

a. Aluminum

Aluminum is the metal that EPA has most recently updated (US EPA 2018) and the only metal for which EPA used the MLR approach to develop the AWQC. Compared to some other metals (e.g., Cu), Al has a relatively small toxicity data set as well as complex environmental chemistry that can strongly influence bioavailability and toxicity (Brix et al. 2020b). A full analysis of the comparison of the chronic Al MLR and BLM is provided in **Appendix D** (Brix et al. 2020b) and

describes the toxicity dataset used for the models. This dataset was an update from the dataset used by Santore et al. (2018) and DeForest et al. (2018 and 2020a; which formed the basis of the MLR used in the EPA AI AWQC). Briefly, chronic MLR models were developed for a representative invertebrate (*Ceriodaphnia dubia*) and fish (*Pimephales promelas*) which both included interactions among TMFs (the *C. dubia* model included a term for interactions between pH and hardness and the *P. promelas* model included terms for interactions between pH and hardness and between pH and DOC. The data used to develop the two species-specific models were pooled to develop a pooled MLR model for the comparison to the BLM. The analysis indicates that the MLR model performs considerably better than the BLM across a range of performance metrics (**Table 3**) and resulted in differences in estimated AWQC as a function of water chemistry.

b. Copper

Copper is the only metal for which the EPA has adopted the BLM approach to develop AWQC (US EPA 2007). Copper has a large toxicity data set over a range of water quality conditions and the environmental chemistry is comparatively simpler than some other metals. A full analysis of the comparison of the acute and chronic Cu MLR and BLM is provided in **Appendix D** (Brix et al. 2020b)². Briefly, six acute species-specific MLR models (four daphnid and two fish) and two chronic models (a daphnid and a fish) were developed and then pooled without interactions for comparison to the BLM. The BLM is the same for both acute and chronic with only the sensitivity adjusted; the MLR models are separate for acute and chronic effects. The analysis indicates that the acute Cu MLR and BLM performance is comparable (**Table 3**), however there are differences in performance on a species-specific basis. In contrast, the chronic Cu MLR performs better than the BLM (**Table 3**). It is important to note that the Cu BLM is optimized for measured Cu accumulations on the biotic ligand and not for toxicity observations (neither chronic nor acute). In contrast, the chronic Cu MLR is based explicitly on chronic Cu toxicity data and so it is not surprising that it performs better than the Cu BLM. For both the acute and chronic modeling approaches, there are differences in the estimated AWQC as a function of water chemistry.

c. Lead

The existing EPA AWQC for Pb are based on a hardness equation (US EPA 1984). A full analysis of the comparison of the acute and chronic Pb MLR models and the BLM is provided in **Appendix E** (DeForest et al. 2020b). Briefly, two acute species-specific MLR models (a daphnid and a fish) and three chronic models (two invertebrates and a fish) were developed and then pooled (separate acute and chronic pooled models were developed) for comparison to the BLM (MLR models without TMF interaction terms were recommended because MLR models with interaction terms resulted in toxicity predictions under some water chemistry conditions that were not mechanistically supported). DeForest et al. (2020b) explains that only the pooled MLR models were compared to the BLM as this approach is most similar where model parameters are

² The copper portion of this analysis has been published and is [available via open access](https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5012) at: <https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5012>

applied to all species. To account for species-specific sensitivity, the sensitivity term varies in the BLM which is similar to the intercept of the pooled MLR model. The analysis indicates that the acute Pb MLR and BLM performance were similar, however the chronic Pb MLR model performs considerably better than the chronic Pb BLM (**Table 3**).

d. Nickel

The existing EPA AWQC for Ni is based on a hardness equation (US EPA 1995). The BLM approach for Ni, described by Santore et al. (2021) in **Appendix F**, provides a critical review of the importance of TMFs (including hardness cations, DOC, and pH) on acute and chronic toxicity to aquatic organisms. The authors also propose a refined BLM that incorporates the conclusions of the critical review of TMFs. The analysis of the “Best Overall Pooled” model clearly shows the broad importance of hardness cations and DOC across all taxonomic groups. A second nickel BLM was developed following the observation that *C. dubia* exhibit poor reproduction at pH > 8, in which the authors propose that these organisms are experiencing combined effects of nickel and bicarbonate toxicities under these circumstances. To address this observation, Santore et al. (2021) developed a species-specific *C. dubia* model which considers both Ni and bicarbonate toxicities. Although this model has only been calibrated and validated with *C. dubia* data, there is preliminary evidence that these effects may be present in other organisms. The refined BLM software contained both the “Best Overall Pooled” BLM and the “North American *C. dubia* BLM”.

The MLR approach, described by Croteau et al. (2021) in **Appendix F**, empirically seeks to explain the influence of TMFs on acute and chronic Ni toxicity. The MLRs account for a similar set of TMFs as the BLMs. Croteau et al. (2021) compares the performance of the BLMs versus the MLRs using a recently published approach for quantifying model performance (Garman et al. 2020; **Appendix A**) and a Ni toxicity and chemistry database consisting of 1498 toxicity observations in 64 studies. The outcome of this comparison is that both models perform similarly, and that both can serve as the basis for normalizing Ni ecotoxicity data for the purpose of developing bioavailability-based AWQC for Ni.

V. Conclusions: Discussion and recommendations of modeling approach

There are now several approaches for modeling metals bioavailability in freshwater. The SETAC Technical Workshop, *Bioavailability-Based Aquatic Toxicity Models for Metals* held in December 2017 sought to assess and provide recommendations on approaches for model development, evaluation, selection, and use. Schlegel et al. (2020) summarized the main recommendations from the workshop and resulting publications: 1) the mechanistic understanding of metal toxicity and speciation should inform all bioavailability models, 2) it is possible to develop simplified tools (including MLR models) that are mechanistically-informed, 3) models should be validated with qualitative and quantitative methods and appropriately applied within a range of water chemistries, and 4) communication regarding the choice of appropriate models, which may be different depending on the situation, needs to be clear. For example, Brix et al (2020b) describe that the selection of the most appropriate model for a given situation requires consideration of several factors including data needs and availability, proposed

model use, model performance, and practical and policy decisions.

In this report, we explored and compared performance of the BLM and MLR approaches for several metals by applying procedures developed as part of the Technical Workshop. Model performance evaluations were conducted for four of the six metals represented by the CRADA. For each metal case study, the BLM and MLR approaches were applied to the same toxicity dataset. In most cases, the empirically-based MLR models performed as well as or better than the mechanistically-based BLM (see **Table 3** and **Appendices D, E, and F**). While there may be metal-specific advantages and disadvantages of using the BLM or MLR approach, it is advantageous, if feasible, for EPA to choose one overarching approach for updating AWQC for all metals. Given the similarities in performance between the BLM and MLR approaches for several metals, with the MLR generally showing somewhat to significantly better performance scores across the acute and chronic metals models examined, and as a practical and policy decision, EPA intends to use MLR models as the overarching metals bioavailability-modeling approach with pH, hardness, and DOC as the core set of TMFs to consider in model development. Additional reasons to recommend the MLR modeling approach are its relative simplicity, transparency, decreased number of input parameters and data collection requirements, ease of use, and reduced need for ongoing maintenance of the models compared to the BLM. However, EPA agrees with Mebane et al. (2020) and Brix et al (2020b) that the development of empirical models like MLR can be informed by mechanistic models like the BLM by helping to identify the key TMFs and expected mechanistic patterns and by evaluating MLR models against existing BLMs.

While MLR models may require lower maintenance than BLMs (Mebane et al. 2020), as EPA moves forward with updating the metals AWQC, it is desirable to have a single software platform. This user-friendly platform would incorporate the updated bioavailability modeling information for all metals so that the user could enter the core set of TMFs once and receive output information on multiple metal criteria values.

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Appendix B

Explanation of How Toxicity Modifying Factors (TMFs) Affect Individual Metals

Aluminum, Copper, Lead, Nickel and Zinc

Developed by CRADA Partners

A. Aluminum

Hardness, pH and DOC

To evaluate how water chemistry affects toxicity, aluminum (Al) HC5 values (hazard concentrations affecting 5% of the population) were calculated by varying DOC, pH, and hardness concentrations. HC5 values were calculated as a function of one parameter being varied and the other 2 held constant. In these examples, HC5s were calculated using the MLR EC20 models and following the USEPA approach. The most noticeable observations are that the HC5 values consistently increase with increasing DOC (Figure 1A–C) and with increasing pH (Figure 1D–F). The influence of hardness on HC5 values is variable depending on pH. Overall, HC5 values increase with increasing hardness at pH 6, remain essentially constant at pH 7, and show a variable pattern at pH 8 (Figure 1G–I). These trends generally follow the empirical data, where available, which is not unexpected given that the MLR models were derived solely from those data. However, fewer empirical toxicity data are available to evaluate the HC5 trends at pH 8. For example, the observation that HC5 values at pH 8 decrease with increasing hardness appears to be consistent with data for *P. subcapitata* but less clearly so for *C. dubia* based on more limited data and insufficient data are available for *P. promelas*. See DeForest et al. 2018 and 2020 for more details.

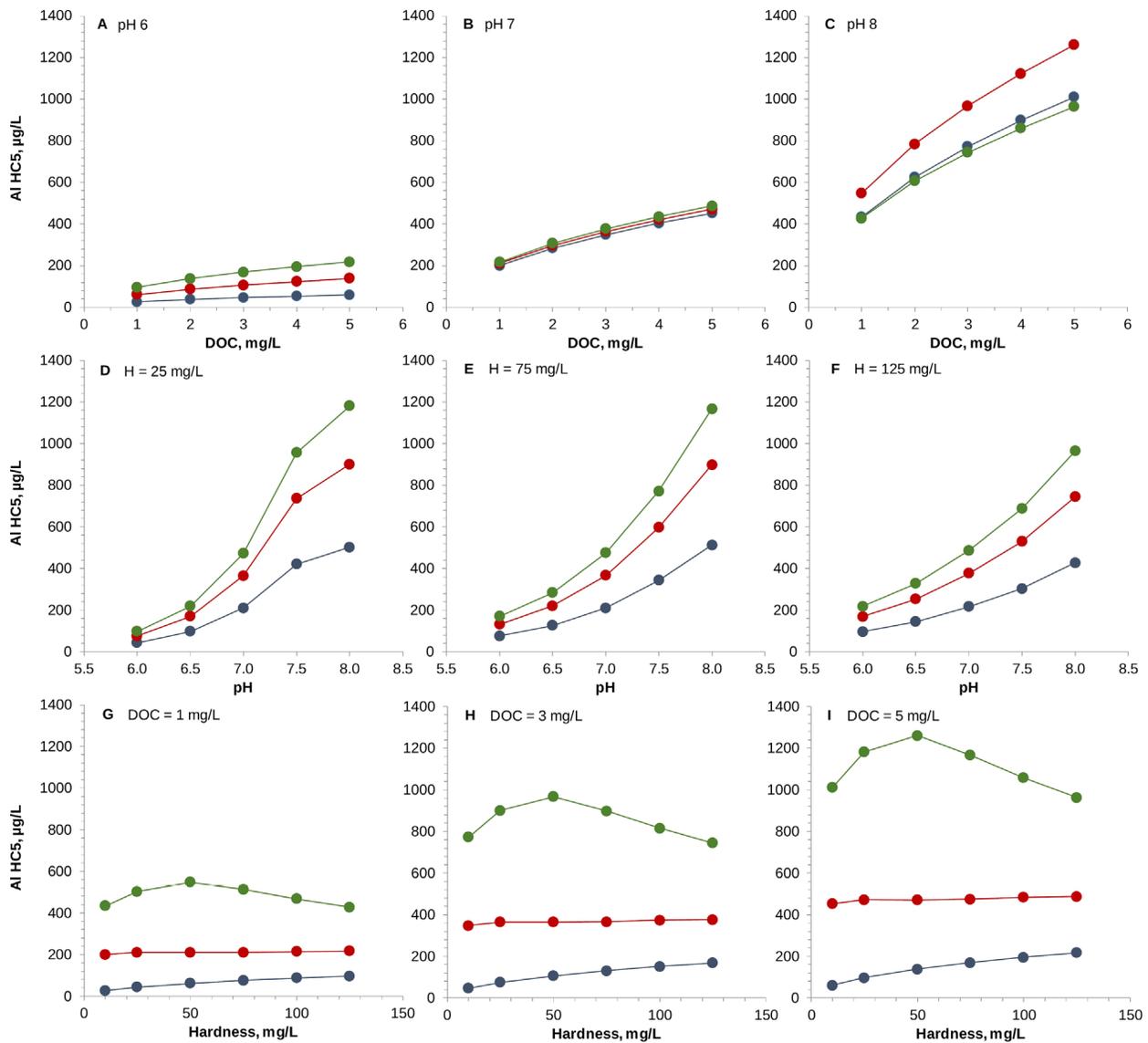


Figure 1: Total Al 5% hazardous concentrations as a function of dissolved organic carbon (DOC) concentration (A–C), pH (D–F), and hardness (G–I). (A–C) Hardness of 10, 50, and 125 mg/L (blue, red, and green symbols, respectively). (D–F) Dissolved organic carbon of 1, 3, and 5 mg/L (blue, red, and green symbols, respectively). (G–I) pH of 6, 7, and 8 (blue, red, and green symbols, respectively). H = hardness; HC5 = 5% hazardous concentration.

Aluminum References

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B. Copper

Hardness

Many studies have reported a protective effect of water hardness on Cu toxicity in acute and chronic exposures to fish and invertebrates (e.g., Waiwood and Beamish 1978; Miller and Mackay 1980; Birge et al. 1983; Winner 1985; Erickson et al. 1996, 1997; Collyard 2002; De Schamphelaere and Janssen 2002; Gensemer et al. 2002; Meyer et al. 2002; Long et al. 2004; Sciera et al. 2004; Van Genderen et al. 2005; Ryan et al. 2009). However, inconsistent results or no protection have been reported in some other studies (e.g., Chapman et al. 1980, Richards and Playle 1999, De Schamphelaere and Janssen 2004b, Hyne et al. 2005, Markich et al. 2005, Wang et al. 2009). Details are provided in Meyer et al. (2007). In terms of the Cu BLM, the hardness effect is characterized by competitive interactions between Cu and hardness cations (i.e., Ca and Mg) at the biotic ligand. For example, \log_{10} values of the biotic ligand binding constants (i.e., $\log K$ values) for both Ca and Mg are 3.60 in the Windward (formerly HydroQual) acute Cu BLM, which is the basis for the U.S. EPA's current acute Cu water quality criteria; and they are 4.40 in the chronic Cu BLMs for fish and invertebrates that were recently proposed by Environment and Climate Change Canada (ECCC) and by the Province of British Columbia (BC). As a complementary approach to the BLM for calculating acute and chronic water quality criteria for Cu, Brix et al. (2017) recently recommended multiple linear regression (MLR) models that included the protective effect of hardness (i.e., represented by a positive regression coefficient for hardness). In the update of these models described in Brix et al. (2020), hardness was also identified as a TMF in all of the MLRs developed.

DOC

In freshwaters, dissolved organic matter (DOM) – quantified as dissolved organic carbon (DOC) – decreases Cu bioavailability and toxicity (e.g., Brown et al. 1974; Lind et al. 1978; Buckley 1983; Winner 1984, 1985; Flickinger 1984; Meador 1991; Oikari et al. 1992; Welsh et al. 1993; Erickson et al. 1996; Hollis et al. 1997; Kim et al. 1999; Ma et al. 1999; De Schamphelaere et al. 2002, 2004, 2006; McGeer et al. 2002; De Schamphelaere and Janssen 2004a, 2004b; Kramer et al. 2004; Schwartz et al. 2004; Sciera et al. 2004; Tusseau-Vuillemin et al. 2004; Van Genderen et al. 2005; Rogevich et al. 2008; Ryan et al. 2009). Details are provided in Meyer et al. (2007). As with other metals, DOC effects are characterized in the Windward, ECCC, and BC Cu BLMs by using a set of discrete binding sites and reactions calibrated in the Windermere Humic Aqueous Model (WHAM; Tipping 1994) in which Cu competes with other metals and cations for binding, thereby decreasing the ability of Cu to bind at the biotic ligand. As a complementary approach to the BLM for calculating acute and chronic water quality criteria for Cu, Brix et al.

(2017) recently recommended multiple linear regression (MLR) models that included the protective effect of DOC (i.e., represented by a positive regression coefficient for DOC). In the update of these models described in Brix et al. (2020), DOC was also identified as a TMF in all of the MLRs developed.

pH

There are several mechanisms by which pH can affect Cu bioavailability, including via speciation, solubility, and competitive interactions between Cu and biotic ligands. Additionally, pH and alkalinity (another water chemistry parameter that can protect against Cu toxicity; Fulton and Meyer 2014, and review in Meyer et al. 2007) usually are positively correlated at pH values exceeding approximately 6.0. Thus, in some experiments with some species, the toxicity of dissolved or total Cu increased as pH was increased; but in other experiments with some species, the toxicity of dissolved or total Cu decreased as pH was increased (e.g., see details in Meyer et al. 2007). However, Cu toxicity to fish and invertebrates, expressed on the basis of Cu^{2+} , increases with increasing pH (e.g., Howarth and Sprague 1978; Meador 1991; Erickson et al. 1996; Collyard 2002; De Schamphelaere and Janssen 2002; De Schamphelaere et al. 2002; Meyer et al. 2002; Ryan et al. 2004, 2009), suggesting the importance of competition between protons and Cu at the biotic ligand. All Cu BLMs incorporate Cu^{2+} speciation in the exposure water (usually via WHAM calculations) and competition with protons at the biotic ligand when predicting Cu toxicity, thus reconciling the otherwise apparently contradictory toxicity results if only dissolved or total Cu concentrations is used to predict toxicity. A generalized bioavailability model (gBAM) can incorporate both the fundamental pH-related speciation effects and the positive relationship between Cu^{2+} toxicity and pH (e.g., Van Regenmortel et al. 2015; Nys et al. 2020). As a complementary approach to the BLM for calculating acute and chronic water quality criteria for Cu, Brix et al. (2017) recently recommended multiple linear regression (MLR) models that included the protective effect of pH [i.e., represented by a positive regression coefficient for pH when calculating dissolved Cu (not Cu^{2+}) criteria]. In the update of these models described in Brix et al. (2020), pH was also identified as a TMF in all of the MLRs developed, with the only exceptions being the species-specific acute *D. pulex* and *O. mykiss* MLRs.

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C. Lead

Hardness

The effect of hardness on Pb toxicity is variable among species and depending on whether acute or chronic exposures were evaluated. In acute Pb exposures, Mager et al. (2011a) observed that hardness (calcium specifically) was protective against Pb toxicity to the fathead minnow (*Pimephales promelas*) but not the cladoceran *Ceriodaphnia dubia*. In contrast, Nys and De Schampheleere (2013) observed that hardness (calcium specifically) did protect against acute Pb toxicity to *C. dubia*. Despite the conflicting data for *C. dubia*, hardness was retained by the Akaike information criterion (AIC) and Bayesian information criterion (BIC) in the MLR models for both *C. dubia* and *P. promelas*, with the hardness slope for *P. promelas* being about two-fold greater than for *C. dubia* (Table 1).³ Hardness was likewise retained by AIC and BIC in the final pooled MLR model for these two species (Table 1).

For chronic Pb exposures, hardness did not have an influence on Pb toxicity to the rotifer *Brachionus calyciflorus* (Nys et al. 2016) nor *C. dubia* (Mager et al. 2011b; Nys et al. 2014). For the snail *L. stagnalis*, *P. promelas*, and the alga *Raphidocelis subcapitata*, the influence of hardness on chronic Pb toxicity was less clear. For *L. stagnalis* and *P. promelas*, series of chronic toxicity tests with only hardness varied were not available for these two species, while for *R. subcapitata* the influence of hardness was equivocal (De Schampheleere et al. 2014). Hardness was retained in the pooled chronic MLR model for invertebrates and fish and in the pooled chronic MLR model for the two most sensitive invertebrates, *C. dubia* and *L. stagnalis* (Table 1).⁴ Ultimately, the final recommended pooled model for chronic toxicity did include hardness, but the influence of hardness was relatively minor compared to dissolved organic carbon (DOC).

DOC

Increasing DOC concentrations consistently reduced both the acute and chronic toxicity of lead (De Schampheleere et al. 2014; Esbaugh et al. 2011, 2012; Mager et al. 2011a,b; Nys et al. 2016; Parametrix 2010). The consistent and, in many cases, strong influence of DOC as a TMF for algal and animal species has led to the development and adoption of a DOC-based bioavailable EQS for Pb in the EU (EC 2010). DOC was retained by AIC and BIC in all MLR models, including the acute individual *C. dubia* and *P. promelas* models and the pooled acute model, as well as in the chronic individual *B. calyciflorus*, *C. dubia*, *L. stagnalis*, *P. promelas*, and *R. subcapitata* models and the pooled chronic model (Table 1).

³ The MLR models referred to in this summary are those that considered hardness, DOC, and pH as individual TMFs, but not interactions of these TMFs, as the final recommended MLR models for lead did not consider interactions.

⁴ For the pooled *C. dubia* and *L. stagnalis* model, AIC retained hardness but BIC did not.

pH

As for hardness, the effects of pH on lead toxicity is variable among species. For acute exposures, increasing pH tends to have a protective effect on lead toxicity to *P. promelas* and a lesser effect on lead toxicity to *C. dubia* (Mager et al. 2011a; Nys and De Schamphelaere 2013). Nevertheless, pH was retained by AIC and BIC in the acute individual species MLR models for *C. dubia* and *P. promelas*, as well as in the pooled acute model (Table 1).

For chronic exposures, from series of tests where only pH was varied, there is evidence that chronic toxicity is reduced with increasing pH for *B. calyciflorus* and *C. dubia* (Nys et al. 2014, 2016). However, pH was not retained by AIC nor BIC in either the individual species MLR models nor in the pooled MLR model (Table 1). Regardless, in selection of the final chronic MLR model, the “full” model with pH included (along with DOC and hardness) was selected based on (1) the empirical data for *B. calyciflorus* and *C. dubia*; and (2) mechanistic support from the biotic ligand model (BLM).⁵ For the alga *R. subcapitata*, the influence of pH is the opposite, with Pb toxicity increasing as pH increases (De Schamphelaere et al. 2014). This is why a pooled MLR model that included both animals and algae was not considered.

Table 1. Summary of TMFs identified in lead MLR models

Exposure	Model	DOC	Hardness	pH
Acute	<i>C. dubia</i>	X	X	X
	<i>P. promelas</i>	X	X	X
	Pooled Acute	X	X	X
Chronic	<i>B. calyciflorus</i>	X		
	<i>C. dubia</i>	X		
	<i>L. stagnalis</i>	X	X	
	<i>P. promelas</i>	X	X	
	Pooled Chronic ¹	X	X	X
	<i>R. subcapitata</i>	X	X	X

¹ The pooled model was based on toxicity data for animals (*C. dubia* and *L. stagnalis*, specifically) because TMFs influence Pb toxicity to algae differently. DOC was retained in the pooled model by both AIC and BIC; hardness was retained by AIC; and pH was included based on considerations from empirical data and mechanisms supported by the BLM.

⁵ As noted in Brix et al. (2020), selection of the final model should not be based solely on strict adherence to statistical methods for model selection, but should also consider mechanistic and other information on how TMFs influence toxicity and validity for other datasets.

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D. Nickel

Hardness

There is a consistent hardness effect on nickel toxicity in acute and chronic exposures to fish and invertebrates (Deleebeeck et al., 2008; Kozlova et al., 2009). In terms of the nickel BLM, this effect is quantified through binding constants (Log K values) of Ca and Mg with the biotic ligand. In the nickel BLM, final Log K values for BL-Ca and BL-Mg were 4.25 and 3.60, respectively. Hardness was identified as a TMF in all available nickel MLR models, with the only exception being the species-specific acute *C. dubia* MLR.

DOC

Increased concentrations of dissolved organic carbon (DOC) consistently shows mitigation of the toxic effects of nickel (Doig & Liber, 2006; Kozlova et al., 2009). DOC effects are simulated in the nickel BLM by using a set of discrete binding sites and reactions calibrated in the WHAM model (Tipping, 1994) in which nickel and other cations in the system can bind to DOC, thereby reducing the ability of the metal to bind at the biotic ligand. DOC was identified as a TMF in all three of the “Pooled” MLRs developed for nickel, in every chronic-species-specific MLR spanning across fish, invertebrates and algae, and identified in both *D. pulex* and *D. pulicaria* acute-species-specific MLRs.

pH

pH effects on nickel toxicity have been observed to be highly species-dependent. While some studies (Deleebeeck et al., 2008; Kozlova et al., 2009; Pyle et al., 2002; Schubauer-Berigan et al., 1993) have shown essentially no change in nickel toxicity to *D. pulex*, *D. magna*, and *P. promelas* in acute exposures ranging from pHs around 5.5 through 8.7, Schubauer-Berigan et al (1993) reported a 10-fold decrease in nickel EC50s between pH 7.3-8.7 in acute exposures to *C. dubia*. The results of this study could be indicative of species-specific differences in pH mechanisms of nickel bioavailability. For the pooled MLR models developed for nickel, only the chronic model identified pH as a TMF. However, pH was identified as a TMF in 7 out of the 10 species-specific nickel MLR models.

Table 1. Summary of TMFs identified in nickel MLR models (adapted from Croteau et al, 2021)

	Model	Duration	Endpoint	Measure	DOC	Hardness	pH
Acute	<i>C. dubia</i>	48h	Survival	LC50			X
	<i>D. magna</i>	48h	Survival	LC50		X	X
	<i>D. pulex</i>	48h	Survival	LC50	X	X	
	<i>D. pulicaria</i>	48h	Survival	LC50	X	X	X
	<i>P. promelas</i>	96h	Survival	LC50		X	X
	Pooled Acute	-	-	-	X	X	
Chronic	<i>C. dubia</i>	7d	Survival + Reproduction	IC25	X	X	X
	<i>D. magna</i>	21d	Reproduction	EC50	X	X	
	<i>D. magna</i>	21d	Survival	LC50	X	X	X
	<i>O. mykiss</i>	17-26d	Survival	LC50	X	X	X
	<i>P. subcapitata</i>	72h	Growth	EC50	X	X	
	Pooled Chronic	-	-	-	X	X	X
Acute + Chronic	Pooled All	-	-	-	X	X	

Nickel References

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E. Zinc

Hardness

There is a consistent effect of hardness on Zn toxicity in acute and chronic exposures to fish and invertebrates (e.g., Hyne et al. 2005; Clifford and McGeer 2009; De Schamphelaere and Janssen 2004; Heijerick et al. 2005). Similar ameliorative effects of hardness have been demonstrated in tests with natural waters where pH was allowed to covary (Mebane et al. 2012). In terms of the Zn BLM, the hardness effect is characterized by competitive interactions between Zn and hardness cations (i.e., Ca and Mg) at the biotic ligand. The biotic ligand binding constants for Ca and Mg for several Zn BLMs, representing multiple organisms and acute and chronic exposures, are summarized in DeForest and Van Genderen (2012). The recently updated MLR-based Canadian water quality guideline (WQG) for Zn, includes hardness as a toxicity modifying factor (TMF) in both the short-term Zn benchmark and the long-term Zn WQG (CCME 2018).

DOC

In freshwaters, dissolved organic matter (DOM) – quantified as dissolved organic carbon (DOC) – generally decreases Zn bioavailability (e.g., Clifford and McGeer 2009; Heijerick et al. 2003), though the effect is not as strong as observed for copper (e.g., Hyne et al. 2005), and mainly at high DOC concentrations (e.g., above 10 mg/L mg/L; Clifford and McGeer 2009; Bringolf et al. 2006). As with other metals, DOC effects are characterized in the Zn BLM by using a set of discrete binding sites and reactions calibrated in the Windermere Humic Aqueous Model (WHAM; Tipping 1994) in which Zn competes with other metals and cations for binding. In the recently updated Canadian WQG, DOC was identified as a TMF for Zn and included as a term in both the short-term benchmark and long-term WQG equations (CCME 2018).

pH

There are several mechanisms by which pH can affect Zn bioavailability, including via speciation, solubility, and competitive interactions between Zn and biotic ligands. Generally, Zn toxicity to fish and invertebrates, expressed on the basis of Zn^{2+} , increases with increasing pH (e.g., De Schamphelaere and Janssen 2004; Van Regenmortel et al. 2017), suggesting the importance of competition between protons and Zn at the biotic ligand. On the basis of dissolved Zn, toxicity generally increases marginally with increasing pH in acute exposures, but the effect is inconsistent in chronic exposures (see summary in CCME 2018), potentially due to differences in bulk solution chemistry characteristics. Santore et al. (2002) describes how differences in bulk chemistry characteristics can influence the relative importance of competitive interactions and speciation on Zn toxicity across a pH gradient. In the recently updated Canadian WQG, pH is included as a TMF in the short-term benchmark, but not the long-term WQG (CCME 2018).

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Addendum

Summary of How Toxicity Modifying Factors (TMFs) Affect Metals Listed in Table 1

Information provided through the peer review by
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Aluminum: Hardness has a moderate role in modifying Al toxicity; pH has a strong role with the greatest toxicity expressed at both low (pH 5) and elevated (pH >8.5) pH, and DOC consistently reduced Al toxicity (DeForest et al. 2018).

Cadmium: Hardness regressions predict acute and chronic toxicity well in natural waters (Mebane 2006; USEPA 2016). pH effects appear weak and ambiguous (Niyogi et al. 2008; Clifford and McGeer 2010). The threshold for a DOC effect appears to be >5 mg/L (Niyogi et al. 2008).

Cobalt: Hardness is clearly important (Diamond et al. 1992; Borgmann et al. 2005). pH at least affected gill uptake, with uptake increasing with increasing pH up to 8.7. DOM reduced Co gill binding, but Co-DOM affinity was much lower than that of Cd, Cu, or Ag (Richards and Playle 1998).

Copper, freshwater: DOC has a strong binding affinity to Cu and predictably reduces Cu toxicity, even at low concentrations (Erickson et al. 1996; Welsh et al. 2008). pH has a strong effect on Cu toxicity, with toxicity tending to decrease with increasing pH in alkaline conditions, but toxicity decreasing with decreasing pH in acidic conditions (Cusimano et al. 1986; Erickson et al. 1996). Hardness is a comparatively minor factor in natural waters (Markich et al. 2005).

Copper, marine: DOC and salinity tend to reduce Cu toxicity in marine and estuarine waters (Grosell et al. 2007; Hall et al. 2008).

Lead: Similar to Cu, DOC and pH have strong effects on the bioavailability and toxicity of Pb (DeForest et al. 2017) Hardness may be an important factor in natural waters, especially when DOC is low (Mebane et al. 2012).

Nickel: Ni toxicity tends to decrease as hardness increased and decrease with increasing DOC. pH has inconsistent influence on toxicity (Croteau et al. 2021; Santore et al. 2021).

Silver: DOC reduces toxicity but pH and hardness influences may be inconsistent (Naddy et al. 2018).

Zinc: Similar to Cd, hardness has a strong influence on Zn toxicity, with decreasing toxicity with increasing hardness (Clifford and McGeer 2009; Mebane et al. 2012; CCME 2018); with fish, toxicity generally increases with increasing pH but relations may be inconsistent in other taxa (De Schampelaere and Janssen 2004). DOC reduces Zn toxicity but some studies suggest influences may be nonlinear, with a threshold of $>\approx 10$ mg/L DOC required to substantially reduce toxicity (Hyne et al. 2005; Bringolf et al. 2006; Ivey et al. 2019).

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