Canadian Environmental Protection Act, 1999

Federal Environmental Quality Guidelines

Copper

Environment and Climate Change Canada

April 2021

Introduction

Federal Environmental Quality Guidelines (FEQGs) provide thresholds of acceptable quality of the ambient environment. They are based solely on the toxicological effects or hazards of specific substances or groups of substances. FEQGs serve three functions: first, they can be an aid to prevent pollution by providing targets for acceptable environmental quality; second, they can assist in evaluating the significance of concentrations of chemical substances currently found in the environment (monitoring of water, sediment, soil and biological tissue); and third, they can serve as performance measures to assess the effectiveness of risk management actions for the chemical substance. The use of FEQGs is voluntary unless prescribed in permits or other regulatory tools. Thus FEQGs, which apply to the ambient environment, are not effluent limits or "never-to-be-exceeded" values but may be used to derive effluent limits. The development of FEQGs is the responsibility of the Federal Minister of Environment under the *Canadian Environmental Protection Act, 1999* (CEPA) (Government of Canada (GC) 1999). The intent is to develop FEQGs as an adjunct to the risk assessment/risk management of priority chemicals identified in the Chemicals Management Plan (CMP) or other federal initiatives. Where data permit, FEQGs are derived following CCME protocols. FEQGs are developed where there is a federal need for a guideline but where the CCME guidelines for the substance have not yet been developed or are not reasonably expected to be updated in the near future. More information on FEQGs is available at https://www.canada.ca/en/health-canada/services/chemical-substances/fact-sheets/federal-environmental-quality-guidelines.html.

This factsheet describes the Federal Water Quality Guideline (FWQG) for the protection of freshwater aquatic life from adverse effects of copper (Table 1) and is based on the biotic ligand model (BLM) approach. The approach allows for the consideration of a diverse set of water quality parameters which are known to impact copper toxicity to aquatic life. The derivation of the FWQG here is largely based on the collection and evaluation of aquatic toxicity data published up to January 2018. No FEQGs have been developed for biological tissue, sediment or soil compartments at this time.

Table 1. Federal Water Quality Guideline for dissolved copper.

Aquatic Life	**Guideline Value (μg/L)**
Freshwater	Variable – Use Biotic Ligand Model[a]
Example FWQG[b]	0.41

[a] The FWQG is for dissolved copper and is calculated using the Biotic Ligand Model Tool (ECCC 2021). The BLM tool and manual can be obtained by emailing: EC.RQE-EQG.EC@Canada.ca

[b] As an example, the site specific copper guideline of 0.41 µg/L is for a water temperature of 20°C, pH of 7.5, DOC of 0.5 mg/L and hardness of 50 mg/L $CaCO_3$.

Substance identity

Copper (CAS 7440-50-8) is a naturally-occurring element commonly found in aquatic systems as a result of both natural and anthropogenic inputs (Nriagu 1979). Natural sources of copper include geological deposits, weathering and erosion of rocks and soils, and volcanic activity. The most important sources of copper in Canada are sulphide minerals such as chalcopyrite ($CuFeS_2$) and bornite (Cu_5FeS_4). In oxic natural waters copper primarily occurs in Cu(II) oxidation state and may form a number of dissolved complexes with either inorganic or organic ligands. Despite constituting a small percentage of the total amount of copper in the water, Cu^{2+} is considered to be the major contributor to Cu toxicity. Cu^{2+} is highly reactive with both inorganic and organic chemicals in solution and in suspension, resulting in a multitude of chemical forms. Copper is a micronutrient and is an essential element to plants and animals (Kapustka et al. 2004). However, at higher concentrations it may become toxic to aquatic life (USEPA 2007).

In Canada, copper is always produced in conjunction with other metals, most often with nickel, zinc, lead, gold and molybdenum. Canada ranked 9th among the 15 top copper-producing countries and ranked 17th for refined copper output in 2014 (NRCan 2015). Canadian mines produced 697,503 tonnes (t) of copper in concentrate in 2014, an increase of 6.9% (652,595 t) from 2013. The increase was mostly attributed to higher production in British Columbia. Most of the Canadian mine production of copper is from British Columbia (47%) and Ontario (30%), followed by Newfoundland (8%), Quebec (6%) and Manitoba (3%). Refineries in Canada produced 325,352 t of refined copper in 2014, an increase of 1.2% (321,511 t) from 2013. Canada's total copper exports in 2014 were $7.1 billion, a 14% increase from 2013 (NRCan 2015).

Uses

Copper is used in electrical wires and cables for its electrical conductivity and in plumbing, industrial machinery and construction materials due to its durability, machinability, corrosion resistance and ability to be cast with high precision and tolerances (NRCan 2015). Because copper does not degrade or lose chemical or physical properties in recycling processes, the International Copper Study Group estimated that in 2012 more than 30% of the world's copper consumption came from recycled copper (ICSG 2014).

Ambient Concentrations

The National Guidelines and Standards Office of Environment and Climate Change Canada (ECCC) compiled the water quality monitoring datasets from ECCC and provinces and territories. The majority of copper monitoring data were for total copper and these data are summarized in Table 2. Total copper concentrations for Canadian jurisdictions varied among jurisdictions with an overall range from 0.002 (Ontario) to 5273 µg/L (Saskatchewan). However, the median (50th percentile) copper concentrations were less variable and ranged from 0.3 µg/L for Prince Edward Island to 1.8 µg/L for Manitoba. Canadian monitoring data for other parameters, including number of sites and samples are presented in ECCC (2021).

Table 2. Ranges and quantiles of total copper concentrations (µg/L) in Canadian surface waters.

Geographic Unit	Minimum	10%	25%	50%	75%	90%	Maximum
Alberta	0.02	0.2	0.5	0.8	1.1	1.9	19
British Columbia	0.02	0.3	0.4	0.7	1.4	3.3	4600
Manitoba	0.2	0.8	1.1	1.8	3.4	6.5	2270
New Brunswick	0.2	0.5	0.5	0.5	0.7	1.0	23
Newfoundland and Labrador	0.02	0.2	0.3	0.4	1.4	2.2	30
Northwest Territories	0.06	0.3	0.5	1.0	2.0	3.7	218
Nova Scotia	0.03	0.2	0.3	0.4	0.8	1.4	158
Nunavut	0.03	0.3	0.7	1.3	2.6	3.8	50
Ontario	0.002	0.4	0.7	1.0	2.1	4.1	2320
Prince Edward Island	0.09	0.2	0.2	0.3	0.5	1.1	3.2
Quebec	0.03	0.4	0.7	1.1	1.5	2.8	25
Saskatchewan	0.02	0.2	0.9	1.5	2.7	8.3	5273
Yukon	0.2	0.4	0.5	0.9	1.8	4.2	46

Mode of Action

As an essential element for life, copper is widely used by plants and animals for the normal functioning of enzymes and proteins (Owen 1982a,b; Linder 1991). Biological functions mediated by copper involve electron transfer catalysis by means of its two accessible oxidation states (Georgopoulos et al. 2002). High levels of copper can be detrimental to aquatic life. Grosell (2011) presents a detailed discussion on mechanism of copper toxicity on fish, including physiological, ion regulatory, behavioral and chemosensory impairments. In plants, elevated copper exposure causes reduced growth, photosynthesis, respiration and nitrogen fixation (Foster 1977; Rosko and Rachlin 1977; Laube et al. 1980).

Fate, Behaviour and Partitioning in the Environment

In nature copper exists in one of four oxidation states: Cu (elemental copper), Cu^+ cuprous ion, Cu^{2+} cupric ion and Cu^{3+} (Georgopoulos et al. 2002). Cupric ion is the most common oxidation state generally encountered in water. Cu^+ is formed from Cu^{2+}; however, it disappears within seconds under oxic conditions (Glazewski and Morrison 1996). Cu^{3+} is strongly oxidizing and occurs only in a few compounds of no environmental significance (ATSDR 2004). Processes that influence the availability of copper to biota are: precipitation, dissolution, adsorption, desorption, complexation and competition for biological adsorption sites (i.e., ligands) (EURAR 2008).

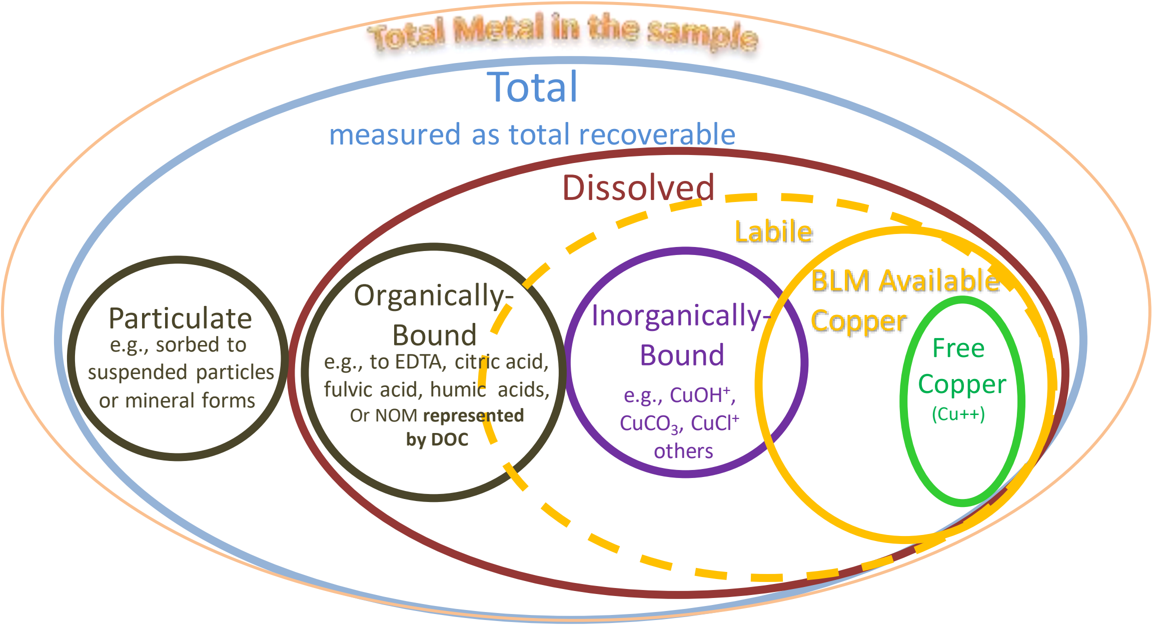

Figure 1: Possible fractions of copper in natural surface waters (Source: Robert Santore, Windward Environmental). NOM: natural organic matter; DOC: dissolved organic carbon.

A schematic of various fractions of copper in surface waters is shown in Figure 1. It may not be possible to measure the absolute total copper concentration in water because of the limitations in routine sampling and analytical methods. The total recoverable copper is often used to represent the total copper concentration. Within the total recoverable fraction, herein referred to as total, both particulate (bound to or incorporated into suspended matter and minerals) and dissolved copper fractions are included. In surface waters, copper can exist as cupric ions (Cu^{2+}), as simple inorganic complexes (e.g., $CuCO_3$) and complexed with organic macromolecules (e.g., bound to humic substances), or may be sorbed onto inorganic and organic particulates (Miwa et al. 1988). Particulate-bound copper is subject to sedimentation. However, the fraction staying within the water column more strongly interacts with the dissolved fraction of total suspended solids (Kiaune and Singhasemanon 2011). The main distinction between particulate and dissolved forms is the filterability, with dissolved copper operationally defined as being able to pass through a filter with pore size of usually 0.45 µm (Paquin et al. 2002). It is important to note that often the dissolved copper fraction is referred as “total dissolved” because it includes metals bound to colloids that are able to pass through the filter, and therefore the dissolved fraction may not be truly dissolved (Paquin et al. 2002). This fraction could also include copper nanoparticles. The possibility that small particles and amorphous precipitates will pass a filter and be quantified as “dissolved” is a limitation of the operational nature of the definition of “dissolved” based on filtrations with a 0.45 µm filter.

The dissolved fraction includes organically-bound, inorganically-bound and free copper ions. Inorganically-bound copper complexes and free copper ions grouped together make up the labile fraction, which is defined by the frequent mobility between the two fractions (Pesavento et al. 2009). Depending on how the labile fraction is determined, it may also include some of the weakly bound organic complexes. The BLM available component is not a specific chemical fraction because bioavailability here refers to the degree to which it elicits toxic responses to exposed organisms, which integrates chemical and biological mechanisms. Generally, the free metal ions are among the most important forms that comprise the bioavailable fraction, being able to cross biological membranes using several transport mechanisms and hence causing toxic effects (Dragun et al. 2008). However for copper, research has shown that both the copper in the form of free ions (i.e., Cu^{2+}) and some simple inorganic complexes (e.g., $CuOH^{+}$) are able to pass biological membranes (Kramer et al. 2004). In contrast, organically-bound copper, presented as dissolved organic carbon (DOC), are considered less bioavailable to organisms (Kramer et al. 2004). Therefore, it is the free copper ion or labile fractions that are the most active copper species. Total copper concentrations are usually not directly related to ecological effects because exposure to organisms is influenced by processes that limit copper uptake (ICPS 1998).

Cu^{2+} typically binds to inorganic or organic ligands when it enters into water and sediments (EURAR 2008). The binding affinity of Cu^{2+} with inorganic and organic matter is dependent on pH and the oxidation-reduction potential in the local environment (EURAR 2008). With increasing pH, the dominant copper species change from Cu^{2+}, $CuCO_3$, $Cu(CO_3)_2^{2-}$, $Cu(OH)_3^{-}$ to ultimately $Cu(OH)_4^{2-}$. The relative proportion of Cu^{2+}, copper hydroxide and carbonate complexes also depends on alkalinity and the magnitude of binding constants for the formation of complexes. Although pH and alkalinity vary substantially in freshwater systems, Cu^{2+}, $Cu(OH)^{+}$, $Cu(OH)_2$ and $Cu(CO_3)_2^{2-}$ make up 98% of dissolved inorganic copper, whereas other oxidation states of copper complexed with hydroxides, carbonates, chlorides, sulphate, ammonium and phosphates comprise <2% of dissolved inorganic copper (Nelson et al. 1986; Alberta Environment 1996).

In surface waters a major proportion of copper can be held in solution through complexation with naturally-occurring organic ligands. In water, copper binds to dissolved organic matter (e.g., humic or fulvic acids) by forming stable complexes with $-NH_2$, -SH and, to a lesser extent, -OH groups (EURAR 2008). Affinity of copper varies among organic ligands, for example, affinity for humic acids is greater than for fulvic acids (Huang and Yang 1995). Cu^{2+} also binds with varying affinities to inorganic and organic components in sediments and soils. For example, Cu^{2+} binds strongly to hydrous manganese and iron oxides in clay and to humic acids, but much less strongly to aluminosilicates in sand (USEPA 2007).

A major fraction of total copper may be adsorbed within the first hour of entering the water column, and in most cases, equilibrium is reached within 24 hours (ATSDR 2004). Adsorption of copper to colloids, suspended particles, sediment and clay plays a major role in the behaviour of copper. The adsorption increases with increasing pH (Nelson et al. 1986; Al-Sabri et al. 1993) and desorption of copper can occur at pH less than 6 (O'Connor and Kester 1975). Organic ligands may increase or decrease adsorption of copper (Davis and Leckie 1978).

Copper that is taken up by fish from water is quickly transferred to blood plasma (Zia and McDonald 1994) and among organs, liver and gills accumulate the most copper (Brungs et al. 1973; Handy 1993; Svobodova et al. 1994). The uptake of copper in fish can vary, less is accumulated in bigger fish compared to smaller fish (Anderson and Spear 1980a,b). In crayfish highest concentrations were reported in the hepatopancreas and gills (Alikhan et al. 1990), whereas in a freshwater mollusk highest copper concentrations were found in gills and mantle (Tessier et al. 1984). Linear uptake of copper was observed in clam and depuration from gills was not observed, while in foot and mantle, concentrations decreased in a linear fashion (Salanki and V.-Balogh 1989). In daphnids reduced copper uptake is reported for waters of high hardness and high humic acid concentration (Winner 1985). The accumulation of copper in green algae initially increases by rapid adsorption followed by a slower intracellular uptake (Knauer et al. (1997). Wilde et al. (2006) reported both extracellular and intracellular copper increases in green algae with increasing copper concentration in water causing growth inhibition.

Bioavailability

Copper in surface waters exists in different chemical forms due to several varying physico-chemical processes and abiotic factors, such as pH, alkalinity, hardness and DOC. Even though copper is present in water in many forms, the toxicity of copper to aquatic life has been shown to be related primarily to activity of the cupric ion, and possibly to some of the hydroxy complexes in fresh water, and bicarbonate complex for plants and algae (Andrew et al. 1977; Chapman and McCrady 1977; Howarth and Sprague 1978; Chakoumakos et al. 1979; Petersen 1982; Borgmann and Ralph 1983; Pagenkopf 1983; Rueter 1983; Allen and Hansen 1996) and marine waters (Zitko and Carson 1973; Sunda and Guillard 1976; Sunda and Lewis 1978; Anderson and Morel 1978). Many examples of classic response of organisms to cupric ion activity, as well as some limited exceptions, are reviewed by Campbell (1995).

A formal description of interactions between metals and organism, now commonly referred to as the Free Ion Activity Model (FIAM), was first provided by Morel (1983). Pagenkopf (1983), using a similar approach, developed the Gill Surface Interaction Model (GSIM) to predict metal effect levels over a range of water quality characteristics. The Biotic Ligand Model (BLM) further formalized these concepts in an easy-to-use tool that automates the calculation of chemical speciation and binding to sensitive receptors in aquatic biota (Playle et al. 1992; DiToro et al. 2001; Santore et al. 2001; Paquin et al. 2002; USEPA 2007). These sensitive receptors are physiologically active sites that correspond to the proximate site of action for toxicity and are collectively termed as the biotic ligand (BL) within the BLM.

Based on the mechanistic principles underlying the BLM, any changes in water quality that would be expected to affect the activity of the free copper ion would be expected to also decrease the bioavailability and hence toxicity of copper. For example, increases in pH, alkalinity and natural organic matter (NOM) would all tend to decrease copper bioavailability and would therefore result in increased copper effect concentrations (e.g., EC_{10s}, or EC_{50s}). The bioavailability of copper can also be modified by competitive interactions at the sensitive receptors (biotic ligand). Increased concentrations of sodium and calcium, for example, can result in reduced binding of copper to physiologically active gill binding sites and can thereby reduce copper bioavailability (Playle et al. 1992). Competition with protons is included in the copper model and could result in lower bioavailability at low pH. But these effects would occur at lower pH values than are typically used in toxicity tests and, as a result, the primary effect of changing pH is to decrease bioavailability at high pH by increasing the OH^- and $CO3^{2-}$ concentrations.

One important feature that is common to the BLM, FIAM and GSIM is the importance of the linkage between bioavailability and the chemical speciation of metals in aqueous systems (Figure 1). All these models assume equilibrium conditions in the water system. Chemical speciation within the BLM is simulated as an equilibrium system that includes complexation of inorganic ions and NOM. The chemical system for the original freshwater BLM is simulated by the Chemical Equilibria in Soils and Solutions (CHESS) model (Santore and Driscoll 1995), which also includes a description of metal interactions with NOM based on the Windermere Humic Aqueous Model (WHAM) (Tipping 1994).

While several versions of WHAM have been developed, the BLM has adopted reactions defined in the development of Model V (Tipping 1994) rather than adopting subsequent versions (e.g., Model VI (Tipping 1998) or Model VII (Tipping et al. 2011)). These latter versions improve the fit of the speciation model to speciation data at very low metal concentrations by increasing the number of strong binding sites that can interact with copper and other metals. While Model VI and Model VII predict copper speciation better than Model V, they are also much more complex and involve three to four times as many reactions as Model V and most of the improvement in predicted speciation occurs at copper concentrations that are lower than values associated with toxic effects. Most of the improvement, therefore, is not relevant in the range of copper concentrations that are of interest to bioavailability modeling. Furthermore, the improvements in Model VI and Model VII result from an increase in strong binding sites, and therefore result in lower estimates of bioavailability for a given copper and DOC concentration. The Model V is therefore selected for developing the FWQG because (i) the model is relatively simple; (ii) the model performs well in the range of concentrations relevant to bioavailability considerations; and (iii) the model produces results that are more protective than Model VI or Model VII.

The BLM includes reactions that describe the chemical interactions of copper and other cations to physiologically active sites (or "biotic ligands") that correspond to the proximate site of action of toxicity. That is, the biotic ligand (BL) is represented by a single characteristic binding site density for the BL and by separate conditional stability constant for each of the dissolved chemical species with which it reacts (Figure 2). Predictions of metal toxicity are made by assuming that the dissolved metal toxicity, which varies with water chemistry, is always associated with a fixed critical level of metal accumulation at the biotic ligand. This fixed level of metal-biotic ligand accumulation at 50 percent mortality (or any other type of biological impairment, such as growth, reproduction or behavior) is referred to as the LA_{50} (or more generally critical accumulation may be more appropriate for a wide range of effects including sublethal chronic effects). It is assumed to be constant, regardless of the chemical characteristics of the site water. This combination of reactions that describe aqueous metal speciation and organism interactions allows the BLM to predict copper toxicity for a variety of organisms over a variety of water quality conditions (Santore et al. 2001).

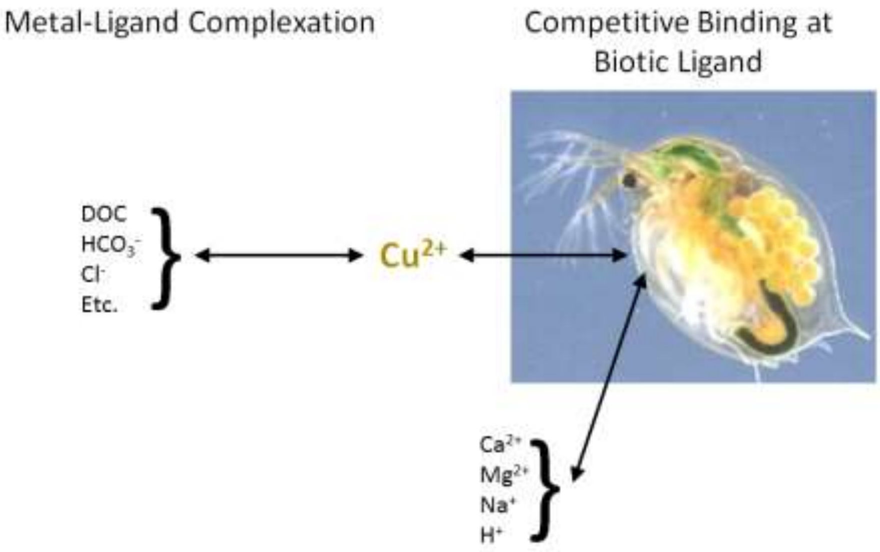

Figure 2. Conceptual diagram showing relationship between chemical speciation and metal complexation, metal binding at Biotic Ligand (BL) sites and competition at these BL sites for other cations.

An advantage of the BLM is that most of the parameters are invariant for different organisms within a specified taxonomic group, despite the complexity of the modeling framework. All thermodynamic constants used to simulate inorganic and organic chemical equilibrium reactions are determined by characteristics of the metal and the available ligands. As such, the constants do not change for simulations involving different organisms within a specified taxonomic group. The ability of the BLM to apply a consistent mechanistic framework to metal bioavailability for organisms has been an important factor in the incorporation of the BLM approach in regulatory frameworks in USA (USEPA 2007) and European Union (EURAR 2008). For example, the US Environmental Protection Agency (USEPA) uses a single set of parameters for all aquatic organisms and in its approach, the copper BLM is used to normalize available toxicity data to a consistent set of exposure conditions. The normalized data are then used to develop a species sensitivity distribution (SSD), and the 5th percentile of the normalized SSD is determined using a truncated triangular distribution (Stephen et al. 1985; Erickson and Stephan 1988). The BLM calculates the final acute value (FAV) as if it was an aquatic organism whose sensitivity to copper was exactly at the 5th percentile (USEPA 2007). The acute water quality criterion is then calculated as the FAV divided by 2, and the chronic criterion as the FAV divided by the acute to chronic ratio (ACR) following the standard guidelines procedures of the USEPA (Stephan et al. 1985).

The BLM modeling framework used by the EU (EURAR 2008) is similar to that used by the USEPA is first used to normalize toxicity data to a consistent set of conditions in order to produce an SSD. However, there are also several differences in the EU approach. First, the toxicity data used by the USEPA are for acute exposures, while the database used by the EU is based on chronic exposures. Second, in addition to fish and invertebrates the EU approach also uses toxicity data for plants, whereas plants are not considered in the USEPA approach. Third, the EU approach uses a log-normal distribution to estimate the 5th percentile (Aldenberg and Jaworska 2000). Finally, the EU approach uses different BLM parameters for fish and invertebrates, and the approach used for plants is a simple regression on free copper ion. The use of different parameter sets in the EU approach requires that the entire SSD be recalculated for each target water body, rather than just calculating the 5th percentile in the USEPA approach.

Federal Water Quality Guideline Derivation

This FWQG is for dissolved copper because (i) dissolved copper is the most bioavailable form; (ii) concentrations of total copper in Canadian surface waters can be affected by non-bioavailable mineral forms; and (iii) most of the toxicity data used for deriving the guideline are based on exposures to dissolved copper. The FWQG also differs from previous guidelines in that it considers the effects of a broad suite of water quality factors on copper bioavailability (i.e., pH, organic matter, alkalinity and ionic content), whereas the existing CCME (CCREM 1987) copper guideline only considered hardness.

The BLM-based FWQG for copper involved compilation and evaluation of freshwater copper toxicity data, evaluation of model performance, utilization of the BLM to normalize the chronic toxicity dataset to site-specific conditions and construction of SSDs (Figure 3). The underlining approach and steps used here are similar to the USEPA (Santore et al. 2001); however, the differences result due to differences in guideline protocols, the parameters considered in the analysis, the details of the toxicity database (e.g., plant data considered, only uses chronic toxicity data) and the normalization of toxicity data in the SSD. Similar to the USEPA, in Canadian BLM a single set of BLM parameters (binding constants) are used for both fish and invertebrate species. Canadian approach is similar to that of the EU in that it is based on chronic toxicity data, considers plants in addition to fish and invertebrates, and utilizes multiple BLM parameters. However, the approach differs from the EU in that it uses a full BLM approach for plants rather than a regression on free copper ion.

The overall data processing steps required to derive the FWQG are shown in Figure 3. In Step 1, individual toxicity endpoints in the database are run through the BLM in speciation mode to calculate critical copper accumulation levels. In this step, the chemistry for the BLM, including temperature, pH, DOC, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, SO_4^{2-}, Cl^{-} and alkalinity, is based on the exposure conditions used in toxicity test and for each toxicity endpoint a critical accumulation value is generated. This step is analogous to finding an intercept specific to a toxicity observation when using a hardness equation. In the case of the BLM, there are two model parameter sets that are used – one for both fish and invertebrates, and the second one for plants (Table 3). For the reactions in Table 3, reactions 2 and 3 represent the formation of bioavailable complexes for $CuOH^{+}$ and $CuHCO_3^{+}$. Because these reactions involve more than two reactants, the log K, logarithm of the binding constant, values are not directly comparable with other reactions in Table 3. For example, the binding of $CuHCO_3^{+}$ to biotic ligand sites appears to be much stronger than the binding of free copper (compare the log K values for reactions 1 and 3), but the magnitude of the log K values cannot be directly compared due to the different stoichiometries of these two reactions. The reactions are written this way because that is the form required by the CHESS model (Santore and Driscoll 1995). To facilitate comparison, these two reactions are written again in an alternative form at the bottom of the table to show an overall log K value useful for comparing with other reactions.

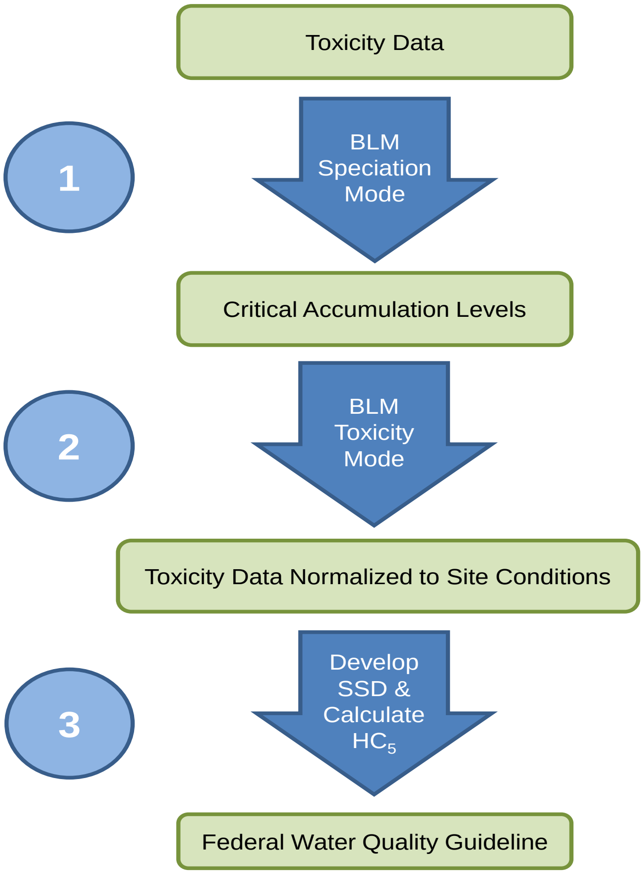

Figure 3. Steps for calculating BLM based FWQG for copper. Each data source or result is shown as green boxes and calculations are shown as numbered blue arrows. Step 1: raw toxicity data are processed with the BLM and the chemistry of the toxicity data exposure conditions to calculate critical accumulation levels. Step 2: critical accumulation levels are processed with the BLM using chemical conditions in the site water to produce normalized toxicity data, expressed as concentrations of copper in the exposure waters. Step 3: normalized concentrations of copper in the exposure waters are used to develop a SSD, and the 5th percentile of the SSD is the FWQG.

In Step 2, normalization process, each of the critical accumulation levels are run with the BLM again, but this time in toxicity mode and with the specified normalized water chemistry (e.g., temperature of 20ºC, pH of 7.5, DOC of 0.5 mg/L and hardness of 50 mg/L). Toxicity mode takes the critical accumulation levels from Step 1 and determines the amount of dissolved copper in the exposure water that would be needed to result in the critical amount of accumulation on the BL of the organism. This needs to be done with the critical accumulation for each toxicity observation in the database, using each set of conditions of site chemistry. The results from this step are normalized concentrations of copper in the exposure waters at the toxicity endpoints. This step is analogous to traditional water quality guideline development when the toxicity data are converted to constant hardness conditions using the hardness slope. Although going from raw toxicity values to normalized toxicity values is often done in one step when using a hardness equation, the BLM needs to do the process in two steps because the BLM contains nonlinearities while the hardness equation is a simple linear relationship. Furthermore, having different parameter sets (log K values) for fish and invertebrates and plants means that the relative positions of organisms in the SSD can change with different chemical conditions, which in turn means that a full database normalization is required for each site's chemistry. At the end of Step 2, the entire toxicity dataset has been normalized to a chosen site chemistry condition. In Step 3, the normalized concentrations of copper in exposure waters are processed into an SSD. For each species and each site, the effect concentrations are averaged (as a geometric mean) by endpoint and effect (only when endpoints are for the same species, same life stage and same duration), and the most sensitive endpoint is then used for that species in the site-specific SSD. The 5th percentile of the best fit SSD model is the site-specific FWQG.

Table 3. Various reactions and log K values used in the BLM to consider bioavailability relationships. One set of values are for plants, and a different set for fish and invertebrates. The log K values are not given for certain reactions because bioavailability relationships for those taxonomic groups do not require specific interactions.

Reaction number	**Reaction**	**log K**		**Compound type**
		Plants	**Fish & inverts**	
1	$\mathrm{BL-Cu = BL + Cu^{2+}}$	5.4	7.4	toxic
2	$\mathrm{BL-CuHCO_3 = BL + Cu^{2+} + H^+ + CO_3^{2-}}$	21.2	-	toxic
3	$\mathrm{BL-CuOH = BL + Cu^{2+} + OH^-}$	-3.3	-0.8	toxic
4	$\mathrm{BL-H = BL + H^+}$	7.2	6.4	competing
5	$\mathrm{BL-Ca = BL + Ca^{2+}}$	4.0	4.4	competing
6	$\mathrm{BL-Mg = BL + Mg^{2+}}$	3.5	4.4	competing
7	$\mathrm{BL-Na = BL + Na^+}$	-	4.0	competing
2 alternate	$\mathrm{BL-CuHCO_3 = BL + CuHCO_3^+}$	9.1	-	toxic
3 alternate	$\mathrm{BL-CuOH = BL + CuOH^+}$	4.2	6.7	toxic

Toxicity data

The EU's chronic aquatic toxicity dataset collected for the risk assessment of copper (EURAR 2008) and the USEPA's data compiled from an extensive copper toxicity data collection and evaluation exercise in 2015 formed the foundation of aquatic toxicity data considered for developing the BLM-based FWQG. ECCC further updated the toxicity dataset through collaboration with the British Columbia Ministry of Environment and Climate Change Strategy. A detailed review of acceptable studies from these sources was also performed by ECCC following the CCME (2007) guidance for data quality. Determinants of test acceptability included, but were not limited to, exposure duration, analytical determination of copper exposure concentrations and other water quality parameters, documentation of the control response, the use of suitable biological endpoints and the inclusion of appropriate statistical analyses of the data collected in the study.

A total of 355 chronic toxicity endpoints for 33 species were identified as acceptable. Many acceptable studies reported results for multiple effects (e.g., reproduction, growth, mortality) with multiple endpoints (e.g., NOEC, LOEC, EC_x). Where multiple effects were available for a single species, only the most sensitive effect was selected following CCME (2007). In an effort to include data for preferred endpoints, if studies did not report an EC_{10}, but reported enough information to develop a concentration-response curve, a response curve was fit to the data and EC_{10} values were calculated using the Toxicity Relationship Analysis Program (TRAP) (USEPA 2015). All 355 acceptable effect concentrations were normalized to temperature of 20ºC, pH of 7.5, DOC of 0.5 mg/L and hardness of 50 mg/L. From the normalized toxicity dataset 83 preferred endpoints for 33 species (12 endpoints for 11 fish species, 39 endpoints for 17 invertebrate species and 32 endpoints for 5 plant species) were selected following CCME (2007). These 83 preferred endpoints along with the test water conditions inputted in BLM are listed in Appendix 1. Finally, geometric means of the effect concentrations of copper in the exposure waters were calculated for individual species if multiple endpoints for same effect and duration were available for them (i.e., one species, one vote).

For each chronic test, temperature, pH, alkalinity, hardness, DOC, %HA (humic acid fraction of DOC), Ca, Mg, Na, K, SO_4 and Cl values were also compiled from studies or estimated. For all studies that required estimation of BLM chemical parameters a method similar to the simple chemistry estimation procedure described in the BLM user manual was used (ECCC 2021). If the chemistry could be inferred from information about the test design or source water, then that inferred chemistry was used (e.g., if the authors reported full chemistry for their control water and then added a known amount of a salt to achieve different levels of hardness). Alkalinity was first estimated when necessary from pH by assuming equilibrium with atmospheric $CO_2(g)$ with a pCO_2 of $10^{-3.2}$ atm. Then, if ion concentrations needed to be estimated from hardness or other ions (e.g., if the authors reported Ca but nothing else), appropriate ion ratios were assigned based on the description of the test water source. Ion ratios were used in the same way as described in the BLM user manual (ECCC 2021). Concentrations of DOC were estimated with the best estimate achievable given the description of the test water, or as 0.3 mg/L in synthetic waters for which values near zero were expected. If toxicity studies reported total copper concentrations instead of dissolved concentrations, the USEPA (1996) conversion factor of 0.96 was applied to convert the total concentrations to dissolved concentrations.

For studies that did not provide any information about the distribution of DOC among humic and fulvic forms it was assumed that 10% of the DOC was in the form of humic acid and the rest as fulvic. This estimate is based on the assumption that most natural waters have a small (but non-zero) amount of humic acid, and that humic and fulvic acids together account for the entire measured DOC. These assumptions are consistent with the way the BLM was used to develop the USEPA criteria for copper (USEPA 2007).

Only 70 of the 355 toxicity observations reported HA fractions, 4 of which were in the SSD. This is not surprising because HA fraction does not typically vary significantly and has a relatively unimportant role in determining toxicity. Excluding instances where the HA fraction needed to be estimated, there were 115 toxicity observations in the database where chemistry needed to be estimated, 40 of which were observations considered for the SSD. Among the 83 endpoints considered for SSD, cation information was estimated for 31, anion information was estimated for 38, DOC values were estimated for 28 and alkalinity was estimated for 7 endpoints.

BLM model validation

The relationship between observed and BLM-predicted copper toxicity for 83 endpoints for 33 species (selected above) was examined to evaluate the performance of the copper BLM (Figure 4). Data from each study were run through the BLM in speciation mode using the test chemistry to get the critical accumulation. A study-specific critical accumulation was determined by taking the geometric mean of the critical accumulations for each study. These critical accumulations along with the test chemistry were run through the BLM in toxicity mode to get the BLM-predicted copper toxicity values. The results showed that the BLM model predicted 92% of the observed toxicity within a factor of 2 for the data used in FWQG. Among the 7 endpoints with greater than the factor of 2 value, 1 was for fish (*Pimephales promelas*), 4 were for invertebrates (*Ceriodaphnia dubia and Villosa iris*) and 2 were for plants (*Chlorella protothecoides*). These results demonstrate that the copper BLM model has performed well.

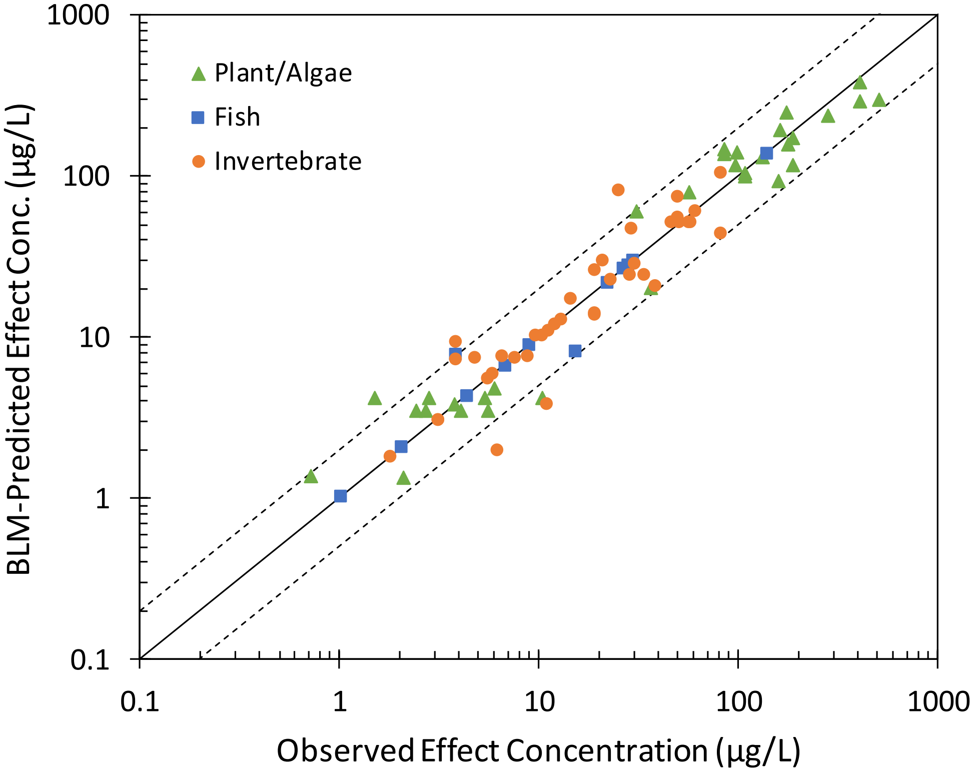

Figure 4. Relationship between observed and BLM-predicted toxicity for the dataset considered for developing FWQG. The solid line represents a 1:1 ratio of observed vs predicted toxicity and the dotted lines represent a factor of +/-2 from measured toxicity. For any data points above the dotted line, the model has a tendency to overestimate the effect-concentration (or underestimate potential Cu toxicity). The reverse is true for data points below the dotted line.

The factor of 2 has been used as a simple and generic way to evaluate the variation between predicted and measured toxicity values and comes from the observation that replicate toxicity tend to be within 2x of the mean (Figure 5). Given the inherent variability in toxicity testing outcomes, even a perfect model could on average only match variation in toxicity values as well as other replicate values, and therefore the boundaries of 2x around a line of perfect agreement shows how close the BLM should match individual observations (Santore et al. 2001). The variation in replicate values was further examined by Santore and Ryan (2015) where it was shown that 2x around the geometric mean corresponds to 1.5 standard deviations around the mean, and this conclusion was supported in a subsequent review by Meyer et al. (2018). With this definition in mind, replicate toxicity values should be within 2x of the mean 88% of the time.

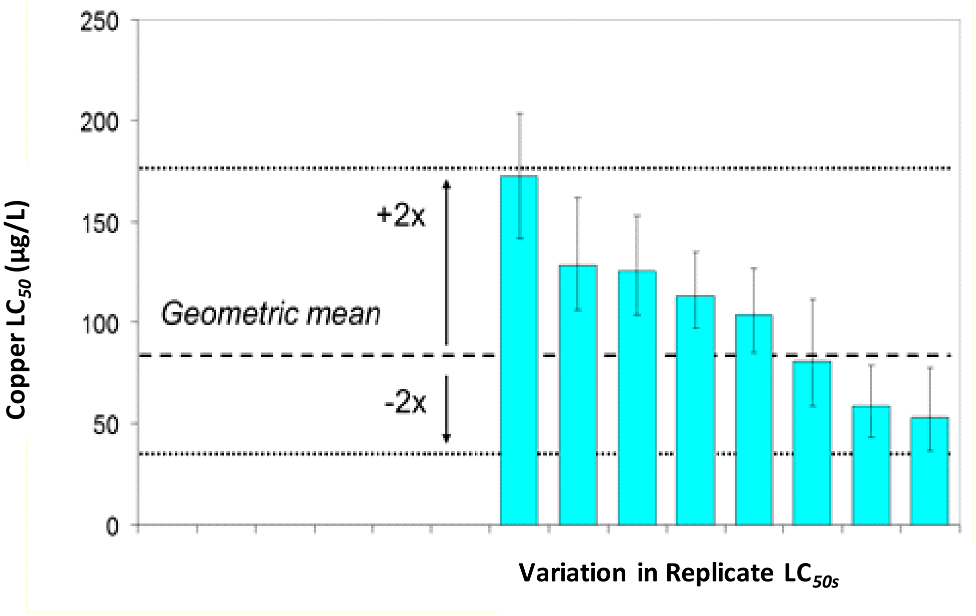

Figure 5. Variation in replicate fathead minnow LC_{50s} in Lake Superior water from Erickson et al. (1987). Replicate values tend to fall within 2x of the geometric mean of all the data.

Guideline derivation

Federal Water Quality Guidelines (FWQGs) are preferably developed using the CCME (2007) protocol. In the case of copper, there were enough acceptable chronic toxicity data to meet the minimum data requirements for a CCME Type A guideline[a]. The CCME (2007) protocol allows for the use of BLM for water quality guidelines, however, no Canadian water quality guideline has yet used this approach. It is to be noted that the BLM based FWQG for copper is for dissolved concentrations of copper. When guideline users only have total copper concentrations for their site, it is recommended that they first compare their total copper concentration to the dissolved copper guideline, and where there is an exceedance, re-sample the waterbody for dissolved copper.

The chronic toxicity dataset and the BLM were combined in a user-friendly BLM tool capable of calculating the site-specific FWQGs. Details of the required steps are given in the user's guide and reference manual (ECCC 2021). In summary, the tool combines all steps of the BLM normalization process and estimation of the 5th percentile from the normalized SSD (as outlined in Figure 3). With the appropriate BLM parameter file, the BLM is applied to the dataset in speciation mode to determine the critical BL-Cu accumulation levels associated with each observation. Parameter files that contain all the thermodynamic constants that define the chemical speciation and metal binding to BL sites (Table 3) are supplied within the BLM tool. The BLM is then used in toxicity mode with the critical BL-Cu accumulation levels and the user-specified normalization chemistry (i.e., the site-specific chemistry). The predicted effect concentrations for the observations included in the SSD thus represent effect concentrations for each observation, as if each test were conducted in the same underlying water

---

[a] CCME (2007) provides two approaches for developing water quality guidelines, depending on the availability and quality of the available data. The preferred approach is to use the statistical distribution of all acceptable data to develop Type A guidelines. The second approach is based on extrapolation from the lowest acceptable toxicity endpoint to develop Type B guidelines. For further detail on the minimum data requirements for CCME guidelines see CCME (2007).

conditions as described by the user-entered site-specific chemistry. Within the BLM tool a geomean value is calculated for species that had multiple endpoints of similar effect and duration. Several cumulative distribution functions are fit to the data using regression methods and the best model is selected based on consideration of goodness-of-fit for each site-specific water chemistry. The 5th percentile value of the best model is the site-specific FWQG (Figure 6).

When the software is used to generate a FWQG for a given water quality condition (i.e. Step 2 in Figure 3), it checks that each of the chemistry input values are within the range of conditions that correspond to the calibration and validation data used in the development and evaluation of the model. When the input value for a chemical parameter is higher than the upper limit, the FWQG is determined with that parameter at the upper limit. Values below the lower limits are similarly replaced with the lower limit before deriving the FWQG.

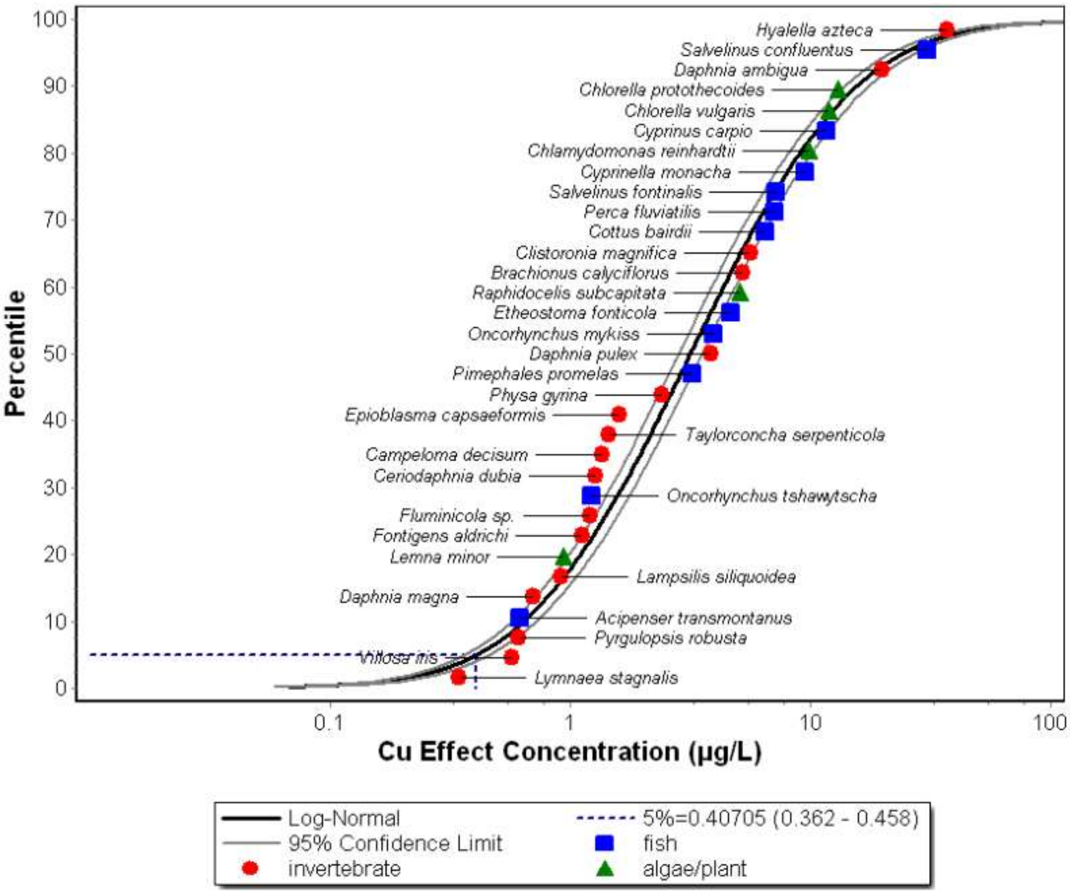

Figure 6. Species sensitivity distribution derived 5th percentile (i.e., guideline) and confidence intervals for effect concentrations (Appendix 1) normalized to temperature of 20ºC, pH of 7.5, DOC of 0.5 mg/L and hardness of 50 mg/L $CaCO_3$. The BLM tool produces a similar SSD plot for the site-specific chemistry inputted by the user (see ECCC 2021).

A FWQG calculator is also provided for users. Since this calculator is intended to be a simple and conservative screening tool, the HC_5 value reported by the look up table is the lowest of the neighboring guideline values. Users are always recommended to use the full BLM software.

Simplified versus full BLM

The BLM tool allows an option to use “simplified” chemistry that uses hardness and geochemical ion ratios to estimate most of the major ions, with alkalinity estimated from pH (ECCC 2021). Using this option reduces the number of input parameters from 10 to 4, and yet produced very similar results for the FWQG (Figure 7). The BLM tool includes average geochemical ion ratios for all of Canada and each ecoregion (ECCC 2021). The tool also allows users to input their own ion ratios for a site. The accuracy of the result that is obtained with the simplified chemistry option can be improved by using geochemical ion ratios that most accurately reflect the site conditions, and therefore it is usually better to use ion ratios at the ecoregional level. For the comparison shown in Figure 7, Canada-wide averages for ion ratios were used and so this result is reflective of the minimal level of agreement that would be expected between the simplified and full chemistry options. Users should use the full chemistry calculation whenever possible, but if data availability does not support the full calculation, the simplified calculation provides an expedient way to overcome data limitations while providing nearly the same results.

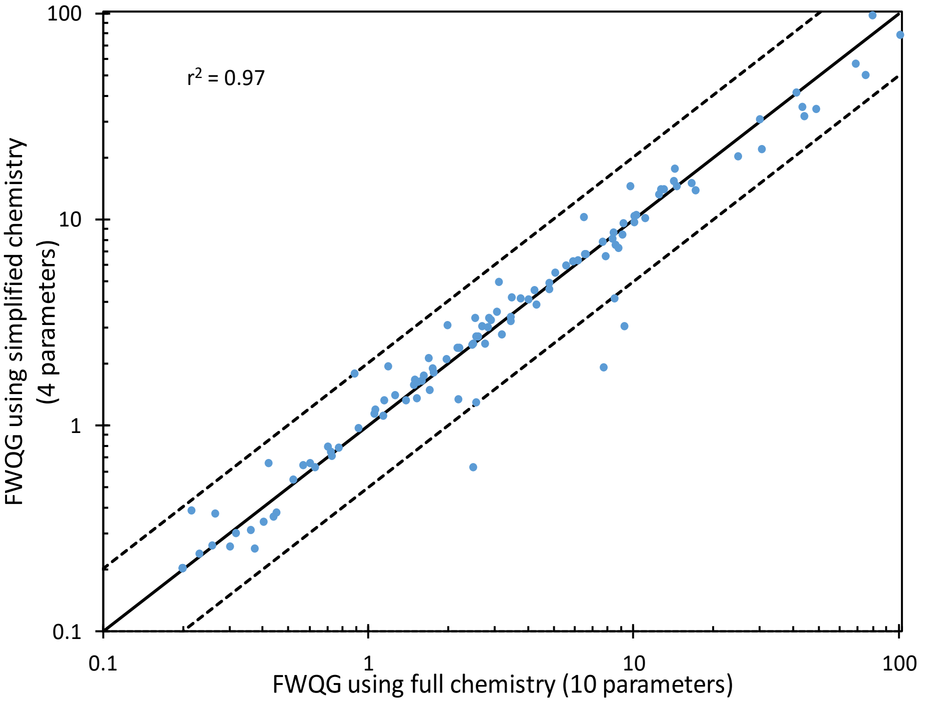

Figure 7. Comparison of FWQGs (μg/L) derived using full and simplified chemistry. Chemistry for this comparison was based on 10 samples selected at random from each of the twelve Canadian Provinces (120 samples in total).

Protectiveness of FWQG

To assess whether the FWQG for copper is sufficiently protective, the BLM-based FWQGs were calculated for each of the 355 acceptable endpoints for 33 species in the chronic toxicity dataset. The ratios of effect concentrations to FWQG were then compared to measured toxicity values at their associated water chemistry (Figure 8). Ratios of >1 indicate that the FWQG is protective of the toxicity value in that particular test, while ratios of <1 indicate that the FWQG is higher than the observed toxicity, and hence may require further evaluation.

The protectiveness assessment identified that 96% of acceptable toxicity data points were protected. To ensure protectiveness, each of the 13 endpoints with ratios <1 were further examined. Only 13 (4%) out of 355 observations fall below the 1:1 line and these endpoints included six endpoints for invertebrates (*Ceriodaphnia dubia* (2), *Villosa iris* (3) and *Daphnia magna* (1)); one for fish (*Acipencer transmontanus*); and six endpoints for plants (*Lemna minor* (3), *Chlorella protothecoides* (2), *Raphidocelis subcapitata* (1)). The mean species ratios for all 33 species in dataset were greater than 1. *Ceriodephnia dubia* endpoints (n=33) had a species mean ratio of 43, meaning on average the reported measured toxicity values were appproximately 43 times higher than the site-specifc FWQG. Similarly, for the other six species a greater number of endpoints had effect concentration to FWQG ratios of greater than one (11 for *V. iris*, 117 for *D. magna*, 4 for *A. transmontanus*, 5 for *L. minor*, 20 for *C. protothecoides* and 43 for *Raphidocelis subcapitata*).

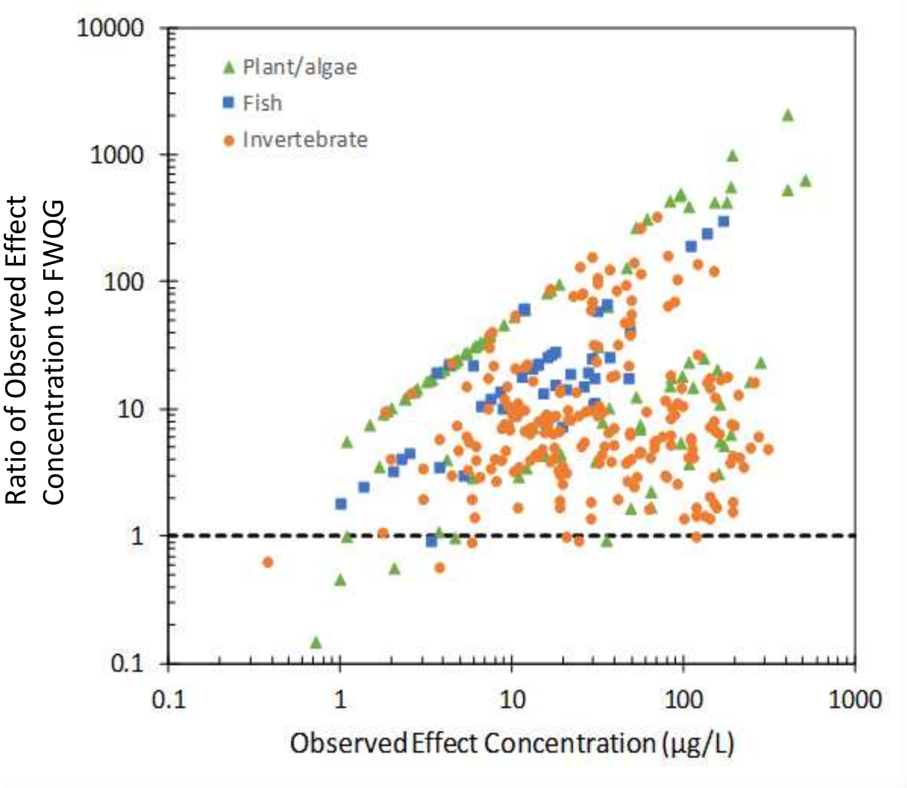

Figure 8. Ratio of observed effect concentrations to FWQG for all acceptable toxicity endpoints plotted against observed effect concentrations.

The FWQG was also compared with a database of olfactory effects in freshwater fish compiled by Meyer and Deforest (2018). In that review, the BLM-based water quality criteria developed by the USEPA (2007) was found to be protective for most (95.3%) of the compiled olfactory toxicity data for copper. The FEQG is generally more protective than the USEPA chronic criterion. Our FEQG results were protective to all of the olfactory toxicity data compiled by Meyer and Deforest (2018).

The overall assessment of the available data as discussed above indicates that the BLM-based FWQG for copper is protective (>95% protection) of aquatic life and thus is in alignment with the derivation of FWQG using the HC_5 of the SSD.

References

Alberta Environment. 1996. Draft water quality guideline for the protection of freshwater aquatic life for copper. Standards and Guidelines Branch, Alberta Environmental Protection, Edmonton, Alberta.

Aldenberg, T. and J.S. Jaworska. 2000. Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicol. Environ. Saf. 46: 1-18.

Alikhan, M.A., G. Bagatto and S. Zia. 1990. The crayfish as a "biological indicator" of aquatic contamination by heavy metals. Water Res. 24: 1069-1076.

Allen, H.E. and D.J. Hansen. 1996. The importance of trace metal speciation to water quality criteria. Water Environ. Res. 68: 42-54.

Al-Sabri, I.Y.N., J.D. Smith and J.D. Thomas. 1993. Copper molluscicides for control of schistosomiasis. 3. adsorption of clay suspensions. Environ. Sci. Technol. 27: 299-303.

Anderson, D.M. and F.M. Morel. 1978. Copper sensitivity of *Gonyaulax tamarensis*. Limnol. Oceanogr. 23: 283-295.

Anderson, P.D. and P.A. Spear. 1980a. Copper pharmacokinetics in fish gills. I. Kinetics in pumkinseed sunfish, *Lepomis gibbosus*, of different body sizes. Water Res. 14: 1101-1105.

Anderson, P.D. and P.A. Spear. 1980b. Copper pharmacokinetics in fish gills. II. Body size relationships for accumulation and tolerance. Water Res. 14: 1107-1111.

Arthur, J.W. and E.N. Leonard. 1970. Effects of copper on *Gammarus pseudolimnaeus*, *Physa integra*, and *Campeloma decisum* in soft water. J. Fish. Res. Board Can. 27: 1277-1283.

Andrew R.W., K.E. Biesinger and G.E. Glass. 1977. Effects of inorganic complexing on the toxicity of copper to *Daphnia magna*. Water Res. 11: 309-315.

Antunes, P.M., M.L. Scornaienchi and H.D. Roshon. 2012. Copper toxicity to *Lemna minor* modelled using humic acid as a surrogate for the plant root. Chemosphere 88: 389-394.

[ATSDR] Agency for Toxic Substances and Disease Registry. 2004. Toxicological profile for copper. U.S. Department of Health and Human Services. Atlanta, Georgia.

Besser J.M., F.J. Dwyer, C.G. Ingersoll and N. Wang. 2001. Early life-stage toxicity of copper to endangered and surrogate fish species (EPA/600/R-01/051). United States Environmental Protection Agency, Washington, DC.

Besser, J.M., N. Wang, F.J. Dwyer, F.L. Mayer, Jr. and C.G. Ingersoll. 2005. Assessing contaminant sensitivity of endangered and threatened aquatic species: Part II. Chronic toxicity of copper and pentachlorophenol to two endangered species and two surrogate species. Arch. Environ. Contam. Toxicol. 48: 155–165.

Besser, J.M., C.A. Mebane, D.R. Mount, C.D. Ivey, J.L. Kunz, I.E. Greer, T.W. May and C.G. Ingersoll. 2007. Sensitivity of mottled sculpins (*Cottus bairdi*) and rainbow trout (*Onchorhynchus mykiss*) to acute and chronic toxicity of cadmium, copper, and zinc. Environ. Toxicol. Chem. 26: 1657–1665.

Besser, J.M., L. Douglas, I. Hardesty, G. Eugene and C.G. Ingersoll. 2009. Sensitivity of freshwater snails to aquatic contaminants: Survival and growth of endangered snail species and surrogates in 28-day exposures to copper, ammonia and pentachlorophenol. Report (CERC-8335-FY07-20-10). U.S. Geological Survey, Columbia Environmental Research Center, Columbia, Missouri.

Besser, J.M., R.A. Dorman, D.L. Hardesty and C.G. Ingersoll. 2016. Survival and growth of freshwater pulmonate and nonpulmonate snails in 28-day exposures to copper, ammonia, and pentachlorophenol. Arch. Environ. Cont. Toxicol. 70: 321-331.

Borgmann, U. and K.M. Ralph. 1983. Complexation and toxicity of copper and the free metal bioassay technique. Water Res. 17: 1697-1703.

Bossuyt, B.T.A and C.R. Janssen. 2004. Influence of multigeneration acclimation to copper on tolerance, energy reserves, and homeostasis of *Daphnia magna* straus. Environ. Toxicol. Chem. 23: 2029-2037.

Brix, K.V., A.J. Esbaugh and M. Grosell. 2011. The toxicity and physiological effects of copper on the freshwater pulmonate snail, *Lymnaea stagnalis.* Comparative Biochemistry and Physiology 154: 261-267.

Brungs, W.A., E.N. Leonard and J.A. McKim. 1973. Acute and long-term accumulation of copper by the brown bullhead, *Ictalurus nebulosus*. J. Fish. Res. Board Can. 30: 583-586.

Campbell P.G.C. 1995. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: Metal Speciation and Bioavailability in Aquatic Systems. Tessier A. and D.P. Turner (eds.). John Wiley and Sons, UK. p. 45-102.

[CCME] Canadian Council of Ministers of the Environment. 2007. A protocol for the derivation of water quality guidelines for the protection of aquatic life 2007. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, 1999, Winnipeg.

[CCREM]. Canadian Council of Resource and Environment Ministers. 1987. Canadian Water Quality Guidelines. Prepared by the Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers.

Chapman, G.A. 1975. Toxicity of copper, cadmium and zinc to Pacific Northwest salmonids. Interim Report Task 002 ROAP 10CAR, Western Fish Toxicology Station, USEPA, Corvallis, OR.

Chapman, G.A. 1978. Toxicities of cadmium, copper, and zinc to four juvenile stages of chinook salmon and steelhead. Trans. Am. Fish. Soc. 107: 841-847.

Chapman, G.A. 1982. Additional toxicity data regarding 1975 chinook salmon early life stage tests with cadmium, zinc and copper (interim report 002 ROAP 10CAR), USEPA, Corvallis, OR.

Chapman, G.A. and J.K. McCrady. 1977. Copper toxicity: a question of form. In Tubb, R.A. (ed). Recent Advance in Fish Toxicology: a Symposium. U.S.EPA Rep. No. EPA 660/3-77/085.

Chakoumakos, C., R.C. Russo and R.V. Thurston. 1979. The toxicity of copper to cutthroat trout (*Salmo clarki*) under different conditions of alkalinity, pH, and hardness. Environ. Sci. Technol. 13: 213-219.

Collvin L. 1985. The effect of copper on growth, food consumption and food conversion of perch *Perca fluviatilis* L. offered maximal food rations. Aquat. Toxicol. 6: 105-113.

Davis, J.A. III, and J. O. Leckie. 1978. The effect of complexing ligands on trace metal adsorption at the sediment/water interface. In: Krumbein, K.E. (ed.). Environmental Biogeochemistry and Geomicrobiology. Vol 3: Methods, Metals and Assessment. Ann Arbor Science. p. 1009-1024.

De Schamphelaere, K.A.C., G. Dagobert, G. Heijerick and C.R. Janssen. 2006. Cross-phylum comparison of a chronic biotic ligand model to predict chronic toxicity of copper to a freshwater rotifer, *Brachionus calyciflorus* (Pallas). Ecotoxicol. Environ. Saf. 63: 189–195.

De Schamphelaere, K.A.C. and C.R. Janssen. 2006. Bioavailability models for predicting copper toxicity to freshwater green microalgae as a function of water chemistry. Environ. Sci. Technol. 40: 4514-4522.

Deaver E. and J.H. Rodgers. 1996. Measuring bioavailable copper using anodic stripping voltametry. Environ. Toxicol. Chem. 15: 1925-1930.

Di Toro, D.M., H.E. Allen, H.L. Bergman, J.S. Meyer, P. Paquin and R.C. Santore. 2001. Biotic ligand model of the acute toxicity of metals. I. Technical basis. Environ. Toxicol. Chem. 20: 2383-2396.

Dragun, Z., B. Raspor and V. Roje. 2008. The labile metal concentrations in Sava River water assessed by diffusive gradients in thin films. Chem. Spec. Bioava. 20: 33-46.

[ECCC] Environment and Climate Change Canada. 2021. Federal Water Quality Guideline for Copper: Biotic Ligand Model (BLM) Tool and User Manual. National Guidelines and Standards Office, Environment and Climate Change Canada, 351 Saint Joseph Boulevard, Gatineau, QC K1A 0H3, Canada. For copy email: EC.RQE-EQG.EC@Canada.ca.

Erickson, R.J. and C.E. Stephan. 1988. Calculation of the final acute value for water quality criteria for aquatic organisms. EPA/600/3-88-018. U.S. EPA, Washington, DC.

Erickson, R.J., D.A. Benoit and V.R. Mattson. 1987. A prototype toxicity factors model for site-specific copper water quality criteria. Revised September 5, 1996. United States Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, MN.

[EURAR] European Union Risk Assessment Report. 2008. Voluntary risk assessment of copper, copper II, sulphate, pentahydrate, copper(I) oxide, copper(II) oxide, dicopper chloride trihydroxide. European Copper Institute (ECI), Tervurenlaan 168, B-1150 Brussels, Belgium.

Franklin, N.M., J.L. Stauber, S.C. Apte and R.P. Lim. 2002. Effect of initial cell density on the bioavailability and toxicity of copper in microalgal bioassays. Environ. Toxicol. Chem. 21: 742–751.

Foster, P.L. 1977. Copper exclusion as a mechanism of heavy metal tolerance in a green algae. Nature 269: 322-323.

Georgopoulos P.G., A. Roy, R.E. Opiekun, P.J. Lioy and M.J. Yonone-Lioy. 2002. Environmental Dynamics and Human Exposure to Copper Volume 1. International Copper Association, Ltd (ed). ISBN 0-943642-12-13.

Glazewski, R. and M. Morrison. 1996. Copper(I)/copper(II) reactions in an urban river. Sci. Tot. Environ. 189/190: 327-333.

[GC] Government of Canada. 1999. Canadian Environmental Protection Act, 1999. S.C., 1999, c. 33, Canada Gazette. Part III, vol. 22, no. 3. Published by the Minister of Justice, Government of Canada.

Grosell, M. 2011. Copper. In: Homeostasis and Toxicology of Essential Metals. Wood, C.M., A.P. Farrell and J. Brauner (eds.). Fish Physiology 31 (Part A): 53-133.

Handy, R.D. 1993. The effect of acute exposure to dietary Cd and Cu on organ toxicant concentrations in rainbow trout, *Oncorhynchus mykiss*. Aquat. Toxicol. 27:1-14.

Hansen, J. A., P.G. Welsh, J. Lipton and D. Cacela. 2002. Effects of copper exposure on growth and survival of juvenile bull trout. Trans. Am. Fish. Soc. 131: 690-697.

Harmon, S.M.,W.L. Specht and G.T. Chandler. 2003. A comparison of the daphnids *Ceriodaphnia dubia* and *Daphnia ambigua* for their utilization in routine toxicity testing in the southeastern United States. Arch. Environ. Contam. Toxicol. 45: 79–85.

Howarth, R.S. and J.B. Sprague. 1978. Copper lethality to rainbow trout in water of various hardness and pH. Water Res. 12: 455-462.

Huang, C. and Y.-L. Yang. 1995. Adsorption characteristics of Cu(II) on humus-kaolin complexes. Water Res. 29: 2455-2460.

[ICPS] International Programme for Chemical Safety. 1998. Environmental Health criteria for copper. World Health Organization Geneva.

[ICSG] International Copper Study Group. 2014. The World Copper Factbook 2014. ICPC, 1000-0013 Lisbon, Portugal.

Kapustka, L.A., W.H. Clements, L. Ziccardi, P.R. Paquin, M. Sprenger and D. Wall. 2004. Issue paper on the ecological effects of metals. U.S. EPA Risk Assessment Forum: Papers Addressing Scientific Issues in the Risk Assessment of Metals. 74 pp.

Kiaune, L. and N. Singhasemanon. 2011. Pesticidal copper (I) oxide: environmental fate and aquatic toxicity. Rev. Environ. Contamin. Toxicol. 213: 1-26.

Knauer, K., R. Behra and L. Sigg. 1997. Adsorption and uptake of copper by the green algae *Scenedesmus subspicatus* (Chlorophyta). J. Phycol. 33: 596-601.

Kramer, K., R. Jak, B. van Hattum, R Hooftman and J. Zwolsman. 2004. Copper toxicity in relation to surface water-dissolved organic matter: biological effects to *Daphnia magna*. Environ. Toxicol. Chem. 23: 2971–2980.

Laube, V.M., C.N. McKenzie and D.J. Kushner. 1980. Strategies of responses to copper, cadmium, and lead by a blue-green and a green alga. Can. J. Microbiol. 26: 1300-1311.

Linder, M.C. 1991. Biochemistry of Copper, Plenum Press, New York. 524 pp.

Meyer, J.S. and D.K. DeForest. 2018. Protectiveness of Cu water quality criteria against impairment of behavior and chemo/mechanosensory responses: an update. Environ. Toxicol. Chem. 37: 1260-1279.

Meyer, J.S., E.M. Traudt and J.F. Ranville. 2018. Is the factor-of-2 rule broadly applicable for evaluating the prediction accuracy of metal-toxicity models? Bul. Environ. Cont. Toxicol. 100: 64-68.

Miwa, T., M. Murakami and A. Mizuike. 1988. Speciation of copper in fresh waters. Anal. Chim. Acta 219: 1-8.

Morel, F.M.M. 1983. Principles of Aquatic Chemistry, Wiley, New York. 446 pp.

Muyssen, B.T.A. and C.R. Janssen. 2007. Age and exposure duration as a factor influencing Cu and Zn toxicity toward *Daphnia magna*. Ecotoxicol. Environ. Saf. 68: 436-442.

Nebeker, A.V., C. Savonen, R.J. Baker and J.K. McCrady. 1984. Effects of copper, nickel and zinc on the life cycle of the caddisfly *Clistoronia magnifica* (Limnephilidae). Environ. Toxicol. Chem. 3: 645-649.

Nelson, H., D. Benoit, R. Erickson, V. Mattson and J. Lindberg. 1986. The effects of variable hardness, pH, alkalinity, suspended clay, and humics on the chemical speciation and aquatic toxicity of copper. EPA/600/3-86/023. PB86-1714444. 132 pp.

[NRCan] Natural Resources Canada. 2015. Minerals and Metals Fact Book – 2015. Natural Resources Canada, Ottawa.

Nriagu, J.O. (ed.). 1979. Copper in the Environment. Part I: Ecological Cycling. 522 pp; Part II: Health Effects. 489 pp. Wiley and Sons, New York.

O'Connor, T.P. and D.R. Kester. 1975. Adsorption of copper and cobalt from fresh and marine systems. Geochim. Cosmochin. Acta. 39: 1531-1543.

Owen, C.A. Jr. 1982a. Biochemical Aspects of Copper: Copper Proteins, Ceruloplasmin, and Copper Protein Binding. Copper in Biology and Medicine Series. Park Ridge, NJ, Noyes Publications. 205 pp.

Owen, C.A. Jr. 1982b. Biological Aspects of Copper: Occurrence, Assay and Interrelationships. Copper in Biology and Medicine Series. Park Ridge, NJ, Noyes Publications. 156 pp.

Pagenkopf, G.K. 1983. Gill surface interaction model for trace-metal toxicity to fish: role of complexation, pH and water hardness. Environ. Sci. Technol. 17: 342-347.

Paquin, P.R., J.W. Gorsuch, S. Apte, G.E. Batley, K.C. Bowles, P.G.C. Campbell, C.G. Delos, D.M. Di Toro, R.L. Dwyer, F. Galvez, R.W. Gensemer, G.G. Goss, C. Hogstrand, C.R. Janssen, J.C. McGeer, R.B. Naddy, R.C. Playle, R.C. Santore, U. Schneider, W.A. Stubblefield, C.M. Wood and K.B. Wu. 2002. The biotic ligand model: a historical overview. Comp. Biochem. Physiol. 133: 3-35.

Petersen, R. 1982. Influence of copper and zinc on the growth of a freshwater algae, *Scenedesmus quadricauda*: the significance of speciation. Environ. Sci. Technol. 16: 443-447.

Pesavento, M., G. Alberti and R. Biesuz. 2009. Analytical methods for determination of free metal ion concentration, labile species fraction and metal complexation capacity of environmental waters: a review. Anal. Chim. Acta 631: 129–141.

Playle, R.C., R.W. Gensemer and G.D. Dixon. 1992. Copper accumulation on gills of fathead minnows: influence of water hardness, complexation, and pH of the gill micro-environment. Environ. Toxicol. Chem. 11: 381-391.

Rosko, J.J. and J.W. Rachlin. 1977. The effect of cadmium, copper, mercury, zinc and lead on cell division, growth and chlorophyll-a content of the chlorophyte *Chlorella vulgaris*. Bull. Torrey Bot. Club 104: 226-233.

Rueter, J.G. 1983. Alkaline phosphatase inhibition by copper: implications to phosphorus nutrition and use as a biochemical marker of toxicity. Limnol. Oceanogr. 28: 743-748.

Salanki, J. and K.V.-Balogh. 1989. Physiological background for using freshwater mussels in monitoring copper and lead pollution. Hydrobiol. 188/189: 445-454.

Santore, R.C. and C.T. Driscoll. 1995. The CHESS model for calculating chemical equilibria in soils and solutions. In: R. Loeppert, A.P. Schwab and S. Goldberg (eds.). Chemical Equilibrium and Reaction Models. Soil Science Society of America Special Publication 42. American Society of Agronomy, Madison, WI. p. 357–375.

Santore, R.C. and A.C. Ryan. 2015. Development and application of a multimetal multibiotic ligand model for assessing aquatic toxicity of metal mixtures. Environ. Toxicol. Chem. 34: 777-787.

Santore, R.C., D.M. Di Toro, P.R. Paquin, H.E. Allen and J.S. Meyer. 2001. A biotic ligand model of the acute toxicity of metals. II. Applicaiton to acute copper toxicity in freshwater fish and Daphnia. Environ. Toxicol. Chem. 20: 2397–2402.

Sauter S., K.S. Buxton, K.J. Macek and S.R. Petrocelli. 1976. Effects of exposure to heavy metals on selected freshwater fish. Toxicity of copper, cadmium, chromium and lead to eggs and fry of seven fish species. Ecological Reseach Series EPA-600/3-76-105. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota.

Stephen, C.E., D.I. Mount, D.J. Hansen, J.R. Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. PB85-227049. U.S. EPA, Washington, DC.

Stouthart, A.J.H.X., J.L.M. Haans, R.A.C. Lock and S.E.W. Bonga. 1996. Effects of water pH on copper toxicity to early life stages of the common carp (*Cyprinus carpio*). Environ. Toxicol. Chem. 15: 376-383.

Sunda, W.G. and R.R Guillard. 1976. Relationship between cupric ion activity and the toxicity of copper to phytoplankton J. Mar. Res. 34: 511–529.

Sunda, W.G. and J.M. Lewis. 1978. Effect of complexation by natural organic ligands on the toxicity of copper to a unicellular alga, *Monochrysis lutheri*. Limnol. Oceanogr. 23: 870–876.

Svobodova, Z., B. Vykusova and J. Machova. 1994. The Effects of pollutants on selected haematological and biochemical parameters in fish. In: R Muller and R. Lloyd (eds). Sublethal and Chronic Effects of Pollutants on Freshwater Fish. FAO Blackwell Science, NY. p. 39-52.

Tessier, A., P.G.C. Campbell, J.C. Auclair and M. Bisson. 1984. Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata* in a mining area. Can. J. Fish. Aquat. Sci. 41: 1463-1472.

Tipping, E. 1994. WHAM - a chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. Computers & Geosciences 20: 973-1023.

Tipping, E. 1998. Humic ion-binding model VI: an improved description of the interactions of protons and metal ions with humic substances. Aquat. Geochem. 4: 3-48.

Tipping, E., S. Lofts and J.E. Sonke. 2011. Humic Ion-Binding Model VII: a revised parameterization of cation-binding by humic substances. Environ. Chem. 8: 225-235.

[USEPA] United States Environmental Protection Agency. 1996. The metals translator: guidance for calculating a total recoverable permit limit from a dissolved criterion. EPA-823-B-96-007.

[USEPA] United States Environmental Protection Agency. 2007. Aquatic Life Ambient Freshwater Quality Criteria - Copper. EPA-822-R-07-001, Washington, DC.

[USEPA] United States Environmental Protection Agency. 2015. Toxicity Relationship Analysis Program (TRAP) Version 1.30a. Mid-Continent Ecology Division, 6201 Congdon Blvd., Duluth, MN 55804.

Wang, N., C.G. Ingersoll, I.E. Geer, D.K. Hardesty, C.D. Ivey, J.K. Kunz, W.G Brumbaugh, F.J. Dwyer, A.D. Roberts, T. Augspurger, C.M. Kane, R.J. Neves and M.C. Barnhart. 2007. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). Environ. Toxicol. Chem. 26: 2048–2056.

Wang, N., C.A. Mebane, J.L. Kunz, C.G. Ingersoll, W.G. Brumbaugh, R.C. Santore, ... and W. Arnold. 2011. Influence of dissolved organic carbon on toxicity of copper to a unionid mussel (*Villosa iris*) and a cladoceran (*Ceriodaphnia dubia*) in acute and chronic water exposures. Environ. Toxicol. Chem. 30: 2115-2125.

Wang, N., C.G. Ingersoll, R.A. Dorman, W.G Brumbaugh, C.A. Mebane, J.L. Kunz and D.K. Hardesty. 2014. Chronic sensitivity of white sturgeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*) to cadmium, copper, lead, or zinc in laboratory water-only exposures. Environ. Toxicol. Chem. 33: 2246-2258.

Wilde, K.L., J.L. Stauber, S.J. Markich, N.M. Franklin and P.L. Brown 2006. The effect of pH on the uptake and toxicity of copper and zinc in a tropical freshwater alga *(Chlorella* sp.). Arch. Environ. Contam. Toxicol. 51: 174–185.

Winner R.W. 1985. Bioaccumulation and toxicity of copper as affected by interactions between humic acid and water hardness. Water Res. 19: 449-455.

Zia, S. and D.G. McDonald. 1994. Role of the gills and gill chloride cells in metal uptake in the freshwater-adapted rainbow trout, *Oncorhynchus mykiss*. Can. J. Fish. Aquat. Sci. 51: 2482-2492.

Zitko, P.W.V. and W.G. Carson. 1973. Prediction of incipient lethal levels of copper to juvenile Atlantic salmon in the presence of humic acid by cupric electrode. Bull. Environ. Contam. Toxicol. 10: 265-271.

List of Acronyms and Abbreviations

ACR – acute to chronic ratio
ATSDR – Agency for Toxic Substances and Disease Registry
BL – biotic ligand
BLM – biotic ligand model
CCME – Canadian Council of Ministers of Environment
CCREM – Canadian Council of Resources and Environment Ministers
CEPA – Canadian Environmental Protection Act
CHESS – chemical equilibria in soils and solutions
CMP – chemicals management plan
DOC – dissolved organic carbon
EC – effect concentration
EU – European Union
EURAR – European Union risk assessment report
FAV – final acute value
FIAM – free ion activity model
FEQG – federal environmental quality guideline
FWQG – federal water quality guideline
GC – Government of Canada
GSIM – gill surface interaction model
HA – humic acid
IC – inhibition concentration
LA – lethal accumulation
LC – lethal concentration
LOEC – lowest-observed-effect concentration
MATC – maximum acceptable toxicant concentration
NOEC – no-observed-effect concentration
NOM – natural organic matter
NRCan – Natural Resources Canada
SSD – species sensitivity distribution
TRAP – toxicity relationship analysis program
USEPA – United States Environmental Protection Agency
WHAM – Windermere humic aqueous model

Appendix 1. Chronic freshwater toxicity data considered for developing the FWQG for copper. Effect concentrations denote as D for dissolved and T for total copper. If studies only reported total copper concentrations, they were converted to dissolve copper using the conversion factor of 0.96 (USEPA 1996). Prior to selecting the individual endpoints from acceptable datasets, reported effect concentrations were normalized for standard temperature (20ºC), pH (7.5), DOC (0.5 mg/L) and hardness (50 mg/L as $CaCO_3$). These normalized effect concentrations are plotted in Figure 4. Test water conditions inputted during normalization in BLM are also listed.

Species	**Group**	**Duration**	**Effect concentration (µg/L)**	**Normalized dissolved effect concentration (µg/L)**	**Temperature (ºC)**	**pH**	**DOC (mg C/L)**	**Hardness (mg/L $CaCO_3$)**	**Reference**
Acipenser transmontanus (White sturgeon)	fish	24 d EC10 (growth)	1.02 D	0.62	15	8.0	0.41	100	Wang et al. 2014
Brachionus calyciflorus (Rotifer)	invertebrate	48 hr EC10 (growth)	7.55 D	5.24	25	6.0	4.91	100	De Schamphelaere et al. 2006
Campeloma decisum (Pointed snail)	invertebrate	42 d EC10 (survival)	6.85 T	1.17	15	7.7	2.65	45	Arthur & Leonard 1970
Campeloma decisum (Pointed snail)	invertebrate	42 d EC10 (survival)	9.21 T	1.56	15	7.7	2.65	45	Arthur & Leonard 1970
Ceriodaphnia dubia (Water flea)	invertebrate	7 d EC10 (reproduction)	11.0 D	4.44	25	8.4	0.40	105	Wang et al. 2011
Ceriodaphnia dubia (Water flea)	invertebrate	7 d EC10 (reproduction)	34.0 D	1.93	25	8.3	3.00	102	Wang et al. 2011
Ceriodaphnia dubia (Water flea)	invertebrate	7 d EC10 (reproduction)	29.0 D	0.78	25	8.3	5.80	106	Wang et al. 2011
Ceriodaphnia dubia (Water flea)	invertebrate	7 d EC10 (reproduction	25.0 D	0.39	25	8.3	10.00	106	Wang et al. 2011
Chlamydomonas reinhardtii (Green algae)	algae/plant	3 d EC10 (growth)	178.0 D	13.90	25	6.0	4.07	250	De Schamphelaere & Janssen 2006
Chlamydomonas reinhardtii (Green algae)	algae/plant	3 d EC10 (growth)	108.0 D	13.15	25	7.0	4.07	250	De Schamphelaere & Janssen 2006
Chlamydomonas reinhardtii (Green algae)	algae/plant	3 d EC10 (growth)	96.0 D	10.19	25	8.1	4.07	250	De Schamphelaere & Janssen 2006
Chlorella protothecoides (Green algae)	algae/plant	72 hr MATC (growth)	1.48 D	9.03	27	7.5	0.30	85	Franklin et al. 2002
Chlorella protothecoides (Green algae)	algae/plant	72 hr MATC (growth)	2.81 D	13.22	27	7.5	0.30	85	Franklin et al. 2002
Chlorella protothecoides (Green algae)	algae/plant	72 hr MATC (growth)	5.31 D	18.90	27	7.5	0.30	85	Franklin et al. 2002
Chlorella protothecoides (Green algae)	algae/plant	72 hr MATC (growth)	10.39 D	27.91	27	7.5	0.30	85	Franklin et al. 2002
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	108.0 D	15.56	25	6.0	2.14	100	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	407.0 D	20.87	25	6.0	6.41	100	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	56.0 D	10.61	25	7.9	2.09	400	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	36.0 D	25.80	25	7.0	0.62	250	De Schamphelaere & Janssen 2006

Species	Group	Duration	Effect concentration (µg/L)	Normalized dissolved effect concentration (µg/L)	Temperature (ºC)	pH	DOC (mg C/L)	Hardness (mg/L CaCO$_3$)	Reference
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	173.0 D	10.40	25	8.0	6.55	400	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	99.0 D	10.53	25	7.0	4.48	250	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	85.0 D	8.23	25	7.0	4.15	500	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	162.0 D	12.66	25	8.8	4.10	250	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	283.0 D	18.03	25	7.1	7.90	250	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	188.0 D	21.15	25	6.0	2.08	400	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	510.0 D	24.48	25	6.1	6.31	400	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	31.0 D	8.45	25	7.9	2.20	100	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	188.0 D	16.38	25	7.9	6.48	100	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	404.0 D	15.80	25	5.5	4.25	250	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	159.0 D	24.38	25	7.1	4.25	250	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	84.0 D	9.05	25	7.0	4.48	250	De Schamphelaere & Janssen 2006
Chlorella vulgaris (Green algae)	algae/plant	3 d EC10 (growth)	132.0 D	15.25	25	7.0	4.24	250	De Schamphelaere & Janssen 2006
Clistoronia magnifica (Caddisfly)	invertebrate	8 month EC10 (life cycle)	5.74 T	5.67	15	7.3	0.70	26	Nebeker et al. 1984
Cottus bairdii (Mottled sculpin)	fish	28 d EC10 (biomass)	26.61 D	6.47	19	8.2	1.00	102	Besser et al. 2007
Cyprinella monacha (Spotfin chub)	fish	30 d IC10 (biomass)	23.0 T	9.50	25	8.3	0.30	162	Besser et al. 2001, 2005
Cyprinus carpio (Common carp)	fish	168 h MATC (survival)	31.0 T	11.71	23	7.6	1.00	100	Stouthart et al. 1996
Daphnia ambigua (Water flea)	invertebrate	10 d EC10 (reproduction)	23.99 T	20.00	21	8.0	0.30	63	Harmon et al. 2003
Daphnia magna (Water flea)	invertebrate	21 d EC10 (reproduction)	61.0 D	4.48	20	7.6	4.00	250	Muyssen & Janssen 2007
Daphnia magna (Water flea)	Invertebrate	21 d EC10 (reproduction)	56.1 D	0.75	20	7.7	5.00	180	Bossuyt & Janssen 2004
Daphnia magna (Water flea)	Invertebrate	21 d EC10 (reproduction)	57.1 D	0.77	20	7.7	5.00	180	Bossuyt & Janssen 2004
Daphnia magna (Water flea)	Invertebrate	21 d EC10 (reproduction)	46.5 D	0.62	20	7.7	5.00	180	Bossuyt & Janssen 2004
Daphnia magna (Water flea)	Invertebrate	21 d EC10 (reproduction)	50.2 D	0.67	20	7.7	5.00	180	Bossuyt & Janssen 2004
Daphnia pulex (Water flea)	invertebrate	42 d NOEC (survival)	4.0 T	1.29	20	8.6	0.30	58	Winner 1985
Daphnia pulex (Water flea)	invertebrate	42 d NOEC (survival)	20.0 T	5.62	20	8.5	0.68	58	Winner 1985

Species	Group	Duration	Effect concentration (µg/L)	Normalized dissolved effect concentration (µg/L)	Temperature (ºC)	pH	DOC (mg C/L)	Hardness (mg/L $CaCO_3$)	Reference
Daphnia pulex (Water flea)	invertebrate	42 d NOEC (survival)	30.0 T	4.81	20	8.7	1.05	58	Winner 1985
Daphnia pulex (Water flea)	invertebrate	42 d NOEC (survival)	5.0 T	2.47	20	8.7	0.30	115	Winner 1985
Daphnia pulex (Water flea)	invertebrate	42 d NOEC (survival)	20.0 T	5.70	20	8.6	0.68	115	Winner 1985
Daphnia pulex (Water flea)	invertebrate	42 d NOEC (survival)	40.0 T	7.89	20	8.6	1.05	115	Winner 1985
Daphnia pulex (Water flea)	invertebrate	42 d NOEC (survival)	10.0 T	3.68	20	8.6	0.38	230	Winner 1985
Daphnia pulex (Water flea)	invertebrate	42 d NOEC (survival)	15.0 T	3.16	20	8.6	0.68	230	Winner 1985
Daphnia pulex (Water flea)	invertebrate	42 d NOEC (survival)	20.0 T	2.76	20	8.6	1.05	230	Winner 1985
Epioblasma capsaeformis (Oyster mussel)	invertebrate	28 d IC10 (survival)	3.1 D	1.61	20	8.2	0.30	162	Wang et al. 2007
Etheostoma fonticola (Fountain darter)	fish	30 d IC10 (survival)	9.3 T	4.64	25	8.1	0.30	163	Besser et al. 2001, 2005
Fluminicola sp. (Pebble snail)	Invertebrate	28 d EC20 (mortality)	11.1 D	1.21	20.35	8.4	1.00	166	Besser et al. 2009, 2016
Fontigens aldrichi (Spring snail)	invertebrate	28 d EC10 (survival)	10.4 D	1.12	20.35	8.4	1.00	166	Besser et al. 2009, 2016
Hyalella azteca (Amphipod)	invertebrate	10 d NOEC (survival)	30.0 D	37.01	20	7.0	0.70	10	Deaver & Rodgers 1996
Hyalella azteca (Amphipod)	invertebrate	10 d NOEC (survival)	82.0 D	74.64	20	7.5	0.70	22	Deaver & Rodgers 1996
Hyalella azteca (Amphipod)	invertebrate	10 d NOEC (survival)	50.0 D	31.91	20	7.7	0.70	36	Deaver & Rodgers 1996
Hyalella azteca (Amphipod)	invertebrate	10 d NOEC (survival)	50.0 D	25.84	20	7.8	0.70	50	Deaver & Rodgers 1996
Hyalella azteca (Amphipod)	invertebrate	10 d NOEC (survival)	82.0 D	30.65	20	8.1	0.70	64	Deaver & Rodgers 1996
Lampsilis siliquoidea (Fatnucket clam)	invertebrate	28 d IC10 (survival)	3.1 D	0.91	20	8.7	0.30	165	Wang et al. 2007
Lemna minor (Duckweed)	algae/plant	7 d EC10 (root length)	2.07 D	2.03	25	8.1	1.00	163	Antunes et al. 2012
Lemna minor (Duckweed)	algae/plant	7 d EC10 (root length)	0.72 D	0.79	25	8.3	1.00	187	Antunes et al. 2012
Lemna minor (Duckweed)	algae/plant	7 d EC10 (root length)	3.78 D	1.37	25	7.8	2.00	35	Antunes et al. 2012
Lemna minor (Duckweed)	algae/plant	7 d EC10 (root length)	5.92 D	1.70	25	7.6	2.00	96	Antunes et al. 2012
Lymnaea stagnalis (Pond snail)	invertebrate	30 d EC10 (growth)	1.8 D	0.34	24	7.8	1.20	56	Brix et al. 2011
Oncorhynchus mykiss (Rainbow trout))	fish	200 hr LC10 (survival)	7.0 T	3.96	12.2	7.2	1.40	24	Chapman 1978
Oncorhynchus tshawytscha (Chinook salmon)	fish	14 weeks EC10 (growth)	2.15	1.23	12.2	7.2	1.40	24	Chapman 1975, 1982
Perca fluviatilis (European perch))	fish	30 d MATC (growth)	29.29	7.13	17.5	7.8	1.00	194	Collvin 1985

Species	Group	Duration	Effect concentration (µg/L)	Normalized dissolved effect concentration (µg/L)	Temperature (ºC)	pH	DOC (mg C/L)	Hardness (mg/L CaCO$_3$)	Reference
Physa gyrina (Pouch snail)	invertebrate	28 d EC10 (growth)	12.0 D	2.39	20	8.3	0.58	170	Besser et al. 2016
Pimephales promelas (Fathead minnow)	fish	30 d IC10 (growth)	4 T	1.56	23	8.3	0.30	162	Besser et al. 2001, 2005
Pimephales promelas (Fathead minnow)	fish	30 d IC10 (growth)	16.0	6.73	25	8.3	0.30	162	Besser et al. 2001, 2005
Pyrgulopsis robusta (Spring snail)	invertebrate	28 d EC10 (growth)	5.9 D	0.61	20.6	8.2	1.10	169	Besser et al. 2009, 2016
Raphidocelis subcapitata (Green algae)	algae/plant	72 hr MATC (growth)	2.69 D	5.46	24	7.5	0.30	15	Franklin et al. 2002
Raphidocelis subcapitata (Green algae)	algae/plant	72 hr MATC (growth)	4.0 D	7.76	24	7.5	0.30	15	Franklin et al. 2002
Raphidocelis subcapitata (Green algae)	algae/plant	72 hr MATC (growth)	2.4 D	4.96	24	7.5	0.30	15	Franklin et al. 2002
Raphidocelis subcapitata (Green algae)	algae/plant	72 hr MATC (growth)	5.5 D	10.23	24	7.5	0.30	15	Franklin et al. 2002
Salvelinus confluentus (Bull trout)	fish	60 d MATC (survival)	138.57 D	30.56	8.5	7.9	0.48	224	Hansen et al. 2002
Salvelinus fontinalis (Brook trout)	fish	30 d EC10 (reproduction)	4.54 T	7.20	10	6.9	0.70	38	Sauter et al. 1976
Taylorconcha serpenticola (bliss rapids snail)	invertebrate	28 d LC20 (survival)	13.0 D	1.44	20.35	8.4	1.00	166	Besser et al. 2009, 2016
Villosa iris (Rainbow mussel)	invertebrate	28 d EC10 (dry weight)	6.2 D	1.91	20	8.3	0.60	101	Wang et al. 2011
Villosa iris (Rainbow mussel)	invertebrate	28 d EC10 (dry weight)	3.8 D	0.24	20	8.3	3.00	98	Wang et al. 2011
Villosa iris (Rainbow mussel)	invertebrate	28 d EC10 (dry weight)	21.0 D	0.42	20	8.2	10.00	100	Wang et al. 2011